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GENETIC DATA ON DROSOPHILA AFFINIS, WITH A DISCUSSION OF THE RELATIONSHIPS IN THE SUBGENUS SOPHOPHORA

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Received February 16, 1940

INTRODUCTION

DROSOPHILA affinis Sturtevant is a member of the subgenus Sophophora (STURTEVANT 1939), closely related to *D. athabasca*, *azteca*, *algonquin*, *narragansett*, and *seminole* (STURTEVANT and DOBZHANSKY 1936b). Cytologically, all the members of this group (*seminole* has not been examined) are aberrant in the subgenus in that they have—in addition to a large V-shaped X chromosome and a small dot-like autosome—three two-armed autosomes (V-shaped or J-shaped—see figure 1). The short arm of one of the J's appears to be wholly heterochromatic, but there remain five autosomal euchromatic arms in addition to the dot—that is, including X and the dot, a total of eight. This was shown by MILLER 1939, for *algonquin;* for *azteca* and *athabasca* this conclusion rests on unpublished information communicated by PROF. T. DOBZHANSKY and MR. E. NOVITSKI. In most other members of the subgenus (*melanogaster*, *pseudoobscura*, *subobscura*, etc.), the total number of euchromatic arms is six. As indicated later in this paper, the European *obscura* probably agrees with the *affinis* group, *ananassae* has an extra X-arm, and in *willistoni*, *earlei*, and their relatives the dot has disappeared. The study of mutant genes in *affinis* was undertaken in order to throw some light on the relation between the distribution of genes in the *affinis* configuration and that in the other members of the subgenus.

MATERIAL

D. affinis occurs in the eastern United States, from central and southern Texas, to southeastern Kansas, the southern shores of the Great Lakes, Massachusetts, and the central portion of the Florida peninsula. Along its northern boundary *affinis* is gradually replaced by *athabasca* (subspecies *mahican*), along its western boundary (at least in Texas) by the ecologically similar *pseudoobscura*—these two species themselves showing a similar replacement along a zone from British Columbia to Colorado (STURTEVANT and DOBZHANSKY 1936b). *D. algonquin* is found along the whole western and northern replacement zone for *affinis*, being present, so far as known, only in areas where *affinis* occurs along with either *pseudoobscura* or *athabasca*. The *affinis-athabasca* replacement zone is also occupied by

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D. narragansett (Woods Hole, Massachusetts; Darien, Connecticut; Ithaca, New York; Wooster, Ohio). *D. seminole* is known only from southern Alabama, deep within the range of *affinis*. It will not be surprising if the ranges of *D. azteca* and *affinis* are found to overlap on one side or the other of the Rio Grande. Thus this species is in contact, or nearly so, with all the other known members of its group, a relation scarcely possible for any of the others, since *seminole* at least seems very unlikely to occur with any of them.

Two subspecies of *D. affinis* have been recognized (STURTEVANT and DOBZHANSKY 1936b), the typical one being lighter in color and more southern in distribution than the subspecies *iroquois*. Study of a large series of living strains from many parts of the range shows that, in fact, southern strains average lighter in color than northern ones. However, both extremes of color may be found throughout the range, and there is no trace of cross-sterility or of any associated differences between the types. The few attempts that have been made to study the inheritance of the difference have not been encouraging. There is so great an age and environmental effect on the character as to make it very difficult to work with. For present purposes it has seemed simplest to ignore the subspecific distinction entirely.

The wild strains used in the present study have come from many regions, and many of them have been collected by friends. I am especially indebted to PROF. J. T. PATTERSON for material from Texas and Louisiana, to PROF. W. P. SPENCER for material from Kansas, Missouri, Tennessee, Ohio, and western New York, and to DR. D. F. POULSON for strains from Maryland and Connecticut. My own collections have been from Massachusetts and Alabama. The wild females have been brought in, and F_2 generations reared from each one separately. In this way a number of mutant types have been isolated; others have appeared in old strains and in experiments concerning the linkage relations of previously found types.

There follows a catalogue of the mutant types studied, arranged by chromosomes, together with a discussion of the possible parallelism of each to types known in other species.

MUTANT TYPES

Left limb of the X chromosome

bobbed. Found in a wild strain originally from Chautauqua, New York. Bristles small, abdominal tergites with rather few bristles and with etched margins. Resembles the bobbed of *melanogaster*, *simulans*, *pseudoobscura*, etc. (see LÜERS 1937). So far as tested, the Y chromosome is without effect on the bobbed character, there being no noticeable sexual dimorphism for it. Y chromosomes from Massachusetts, New York, Tennessee, and Kansas have been studied. Bobbed females have been sterile so far as tested.

cut. Found in a wild strain originally from Woods Hole, Massachusetts. Wings with nicks in margin; often extreme, sometimes overlapping wild type, especially in females. Often the humeri are more separate from the mesonotum than usual, suggesting epaulets. Except in this last respect the character is much like the beaded of *pseudoobscura*, and also resembles cut of *melanogaster*.

pebbled. Found in experiments with a cut vermilion chromosome. Wing surface coarsely granular. Often of low viability and fertility, but is easily kept in one homozygous strain that apparently happens to have modifiers favorable for it.

scute-1. Arose in linkage experiments. Resembles the scutes of *melanogaster*, scutellar of *pseudoobscura*, etc. Both scutellar bristles are usually absent, and the anterior and middle orbitals are often absent. In some cultures a dorsocentral is also missing. The character is variable, and sometimes overlaps wild type, especially in females.

scute-2. Arose in experiments involving scute-1, and was at first confused with it. Resembles scute-1, but is less viable and fertile, and less frequently affects the dorsocentral bristles.

stubby. Found in linkage experiments. Bristles slightly shortened and curved. Perhaps a slight allele of either forked or singed (*melanogaster*, *simulans*, *pseudoobscura*, and other species).

vermilion. Arose in the cut stock. Quite similar to the vermilion of *melanogaster* and other species. MR. C. W. CLANCY tested it for me by the feeding technique of BEADLE and LAW (1938), and reports that it lacks the v^+ substance that is present in wild-type *affinis* (vermilion brown *melanogaster* larvae were used as test material). There can therefore be no doubt of the identity of the locus.

white-1. Arose in the same culture as scute-1, but in the other X of the mother of that culture. Eyes completely white, as in the well-known types of *melanogaster* and other species.

white-2. Arose in a scute-1 scarlet chromosome. Completely white eyes, allelic to white-1.

Right limb of the X chromosome

ascute. Two males were found in a linkage experiment. Resembles ascute and grooveless of *melanogaster* or of *pseudoobscura*.

hairy. Found in a strain from Gatlinburg, Tennessee. There are a few hairs on the dorsal surface of the scutellum. The character is variable; often only one hair is visible, and sometimes the specimens look wild type. In many crosses some of the heterozygous females have one or two of these hairs present. The resemblance to hairy of *melanogaster* would be of doubtful significance were it not in agreement with the other parallels in this arm.

hairy-2. Found in linkage experiments with cut and scarlet. It is indistinguishable from hairy-1, and allelic to it.

scarlet. Arose in a cut vermilion stock, where its presence was only detected as a result of apparent inconsistencies in linkage experiments, since vermilion, scarlet, and vermilion scarlet are indistinguishable in eye color. The resemblance to scarlet of *melanogaster* or *pseudoobscura* is clear.

sex-ratio. Found frequently in wild strains from various localities (Woods Hole, Massachusetts; Coffeyville, Kansas; Gatlinburg, Tennessee —see STURTEVANT and DOBZHANSKY 1936a). This gene is not easy to study in *affinis*, since its expression is variable; males carrying it often give essentially normal sex-ratios, yet descendants carrying the same X may give large families with few or no sons. For this reason statistics concerning the frequency of the gene in wild populations are of little value. Sex-ratio has been found associated with only one of the two sequences referred to below (the inverted one); it remains uncertain whether it is always present in chromosomes having that sequence.

tilt. Found in the original white-1 chromosome. The wings are often curved upwards slightly at their tips, but a more useful index is the presence of extra veins between the second and third longitudinal veins at about the level of the anterior crossvein, the two longitudinals often being fused here into a knot. The character is variable, frequently overlapping wild type, especially in females. Resembles tilt of *melanogaster*.

veinlet. Found in early experiments with white-1, of the same series of cultures as that in which tilt was found. The third, fourth, and fifth longitudinal veins are often incomplete at their distal ends. Overlaps wild type. Resembles short of *pseudoobscura*, or veinlet of *melanogaster*, though it is less extreme than the latter.

Second chromosome

abnormal. Found in a strain from Baltimore, Maryland. Abdominal bands irregular, tergites often failing to meet in the mid-dorsal line. Overlaps wild type.

arc. Found in a wild stock from Woods Hole, Massachusetts. Wings rather short, bowed as in the arc of *melanogaster*, but with no approximation of the crossveins. Overlaps wild type.

bithorax. Found in linkage experiments with chromosome 5. The balancers are wing-like. Overlaps wild type. Resembles some of the alleles of bithorax in *melanogaster* or *pseudoobscura*.

claret. Present in a wild strain from Baton Rouge, Louisiana. Resembles pinkish and pink-like of *affinis*, pink and claret (as well as other eye colors) of *melanogaster*, *simulans*, or *pseudoobscura*.

crossveinless. Found in the stock of pinkish, of pure Woods Hole ancestry. Posterior crossvein broken or absent. Overlaps wild type frequently. Resembles crossveinless-d of *melanogaster;* not like crossveinless of *melanogaster* (X) or of *pseudoobscura* (2).

crumpled-1. Found in F_2 from a wild female from Litchfield, Connecticut. Wings abnormal, distal part often folded back under proximal part; branches of arista bent anteriorly near their bases, so that distal parts are parallel to main axis; bristles blunt, somewhat shortened; fertility greatly reduced. Resembles crumpled of *melanogaster* or *pseudoobscura*.

crumpled-2. Found in linkage experiments. Phenotypically not distinguishable from crumpled-1; allelism and linkage not tested.

Delta. Found as two individuals in a linkage experiment. Dominant. Veins thickened, especially at junctions, acrostichal hairs numerous and in irregular rows, eyes somewhat roughened, tarsi short and thick. Lethal in homozygous condition. Resembles Delta of *melanogaster* or *simulans* and Smoky of *pseudoobscura*.

knot. Found in a strain from Woods Hole. Slight thickening of junctions of veins. Overlaps wild type. Not easily classified.

pinkish. Found in F_2 from a wild female from Woods Hole, Massachusetts. Pink eye color, resembling pink, claret, ruby, garnet, etc. of *melanogaster*, or pink, claret, magenta and ruby, of *pseudoobscura*.

pauciseta. In a wild stock from Gatlinburg, Tennessee. Many of the bristles and hairs are reduced in size or absent, in an irregular pattern. Often so nearly wild type as to be difficult to classify. Resembles pauciseta of *pseudoobscura*, and may also be compared to recessive hairless of *simulans*.

rugose. Found in F_2 from a wild female from Lake of the Ozarks, Missouri. Eyes distinctly rough, of normal size and convexity.

short-wing. In the wild stock from Woods Hole, Massachusetts that contained knot. The two genes were originally associated in the same chromosome. Wings short, blunt, of uneven texture. Variable, and sometimes overlaps wild type.

short-1. Found in linkage experiments. Character similar to veinlet, but somewhat more extreme and less often overlapping wild type.

short-2. Found in F_2 from a wild female from Gatlinburg, Tennessee. Not different phenotypically from short-1, to which it is allelic.

short-tarsi. Found in F_2 from a wild female from Mendham, New Jersey. Legs, especially tarsi, short. Often a strong constriction in the tarsi, leaving the terminal segments attached to the rest of the legs by a thread-like connection. Arista often with a thickened axis.

tiny-bristle. Found in a stock from Wooster, Ohio. Bristles fine, not shortened. No marked effect on development rate.

Third chromosome

cinnabar. Present in a wild strain from Austin, Texas. Bright eye color, not separable from vermilion or scarlet. Feeding experiments made for me by MR. D. D. MILLER show that it has v^+ but not cn^+ substances, that is, that it corresponds to cinnabar of *melanogaster* and orange of *pseudoobscura*.

narrow. Found in linkage experiments. Wings narrow, cross veins approximated; legs rather short; head and mesonotum rather short and broad, sometimes slightly concave in mid-dorsal line. Resembles lanceolate of *melanogaster*, or narrow of *pseudoobscura*.

pink-like. Found in F_2 from a wild female from Woods Hole, Massachusetts. Like pinkish, but slightly more extreme. This, like pinkish, gives, when combined with vermilion, a clear yellow eye-color.

roughish. Found in a strain from Coffeyville, Kansas. Eyes rough, often somewhat wrinkled; various head and thoracic bristles may be absent; variable, sometimes quite extreme but may overlap wild type.

straw. Found in linkage experiments. Yellowish body-color, quite similar to the straw of *melanogaster* or of *simulans*.

Fourth chromosome

antennipedia. Found in F_2 from a wild female from Litchfield, Connecticut. The antennae are more or less leg-like, varying from normal antennae up to an extreme in which a practically complete leg (femur, tibia, basal tarsal segments), bearing a rudimentary arista at its tip, is attached to the rudimentary base of the third antennal segment. The type differs radically from the aristapedia of *melanogaster* or *simulans*, where the normal antenna becomes more or less leg-like—the second antennal segment becoming femur-like, the third tibia-like, and the arista quite tarsus-like. In antennipedia all the normal antennal parts persist, including an arista (which may be short, thick, or rudimentary, but is inserted at the normal position), while the apex of the third segment has some portions of a leg attached to it. Another property of antennipedia is that, in the males, sex-combs are often present on the tarsi of the second and third pairs of legs (more often the second) as well as the first where they normally occur. This applies more particularly to the longer sex-comb of the basal tarsal segment; the single tooth of the comb on the second segment is sometimes present on the second leg, but has not been seen on the third.

jaunty-1. Found in F_2 from a wild female from Gatlinburg, Tennessee. Resembles jaunty of *melanogaster* or of *pseudoobscura*, curled of *melanogaster*, upturned of *pseudoobscura*, etc.

jaunty-2. Found in F_2 from a wild female from Mendham, New Jersey. Like jaunty-1 and allelic to it.

jaunty-3. Found in F_2 from a wild female from Baton Rouge, Louisiana. Like jaunty-1 and allelic to it.

TABLE 1

Corresponding mutant types.

affinis	*pseudoobscura*	*melanogaster*
XL	XL	X
bobbed	bobbed	bobbed
cut	beaded	cut
miniature	miniature	miniature
scute	scutellar	scute
vermilion	vermilion	vermilion
white	white	white
yellow	yellow	yellow
XR	XR	3L
ascute	ascute	ascute
hairy	—	hairy
scarlet	scarlet	scarlet
sex-ratio	sex-ratio	—
tilt	tilt	tilt
veinlet	short	veinlet
2	2	3R
bithorax	bithorax	bithorax
claret	pink, claret	pink, claret
crossveinless	—	crossveinless-d
crumpled	crumpled	crumpled
Delta	Smoky	Delta
pinkish	pink, claret	pink, claret
pauciseta	pauciseta	(Hairless ?)
3	3	2R
cinnabar	orange	cinnabar
narrow	narrow	lanceolate
straw	—	straw
4	4	2L
jaunty	jaunty	jaunty
net	tangled	net
truncate	—	dumpy
5	5	4
abdomen rotatum	—	abdomen rotatum
reduced	—	(shaven ?)

net. Present (heterozygous) in a wild male from Ithaca, New York. Extra wing-veins, especially between the distal ends of the second and third veins, and around the posterior crossvein. Resembles net of *melanogaster*, or plexus and tangled of *pseudoobscura*.

truncate. Found in linkage experiments. Wings short, often with the characteristic "truncate" shape, that is, strongly indented at the posterior distal corner, but with intact margin. Overlaps wild type. Resembles some of the dumpy alleles of *melanogaster*

Fifth chromosome

abdomen rotatum. Found in linkage experiments. The abdomen is twisted about its longitudinal axis in a counterclockwise spiral. Both sexes are sterile. Resembles the abdomen rotatum of *melanogaster*.

Fused. Arose in a cross between a strain from Woodstock, Maryland, and one that carried several mutant genes. Dominant, lethal when homozygous. Third and fourth veins strongly convergent at their distal ends; ocelli and ocellar bristles usually absent. Resembles the fused of *melanogaster*, except that that type is recessive and has a peculiar type of lethal action (LYNCH 1919) that is not evident in *affinis*.

reduced. Found in a strain from Woodstock, Maryland. Posterior scutellar bristles (occasionally also the anterior ones, rarely a few other thoracic bristles) very short, thick, and blunt. Suggests the shaven of *melanogaster*, though not a convincing parallel.

CHROMOSOME MAPS

The X chromosome

Two different sequences occur in each arm, the "standard" sequence of XL being regularly associated with the standard of XR, and the inverted sequences being likewise associated. Both sequences have been found in strains from Woods Hole, Tennessee, Missouri, Kansas, and Texas. The association between the two limbs remains unexplained, but can probably be interpreted when salivary gland preparations are studied.

Scute-1, white-2, and hairy-1 arose in the inverted sequence; scute-2, white-1 and hairy-2 in the standard. Ascute, tilt, and scarlet all arose in standard, but were later transferred to the inverted sequence by crossing over. These six genes are therefore known in both sequences; the others only in one or in the other. Maps are shown for each sequence.

The maps are unsatisfactory, since many of the mutant types are unsuited to exact linkage studies. Pebbled, bobbed, scute-2, and ascute all have markedly low viability; cut, both scutes, both hairys, tilt, and veinlet, all overlap wild-type; and white, vermilion, and scarlet interfere with each other's classification.

In females heterozygous for the two sequences there is very little crossing over, not over one percent for the whole known region. Most or all of that which occurs is in the region to the right of hairy. Patroclinous sons have been found rarely from such females, but it is not certain that they

were XO in constitution, so no definite conclusions can be drawn from them.

Perhaps the most unusual relation shown here is that yellow and scute are not close to each other as in most species, but are separated by cut, vermilion, and pebbled.

TABLE 2

Linkage maps of X, second and fourth chromosomes of D. affinis.

STANDARD SEQUENCE		INVERTED SEQUENCE	
stubby	0	miniature	0
white	2	scute	6
yellow	12	white	12
cut	17	hairy	28
vermilion	22	veinlet	40±
pebbled	24	tilt	40±
scute	27	ascute	60
bobbed	40±	scarlet	90
hairy	40±		
veinlet	50±		
tilt	50±		
ascute	60		
scarlet	99		

SECOND CHROMOSOME

pauciseta	0
short-wing	1±
rugose	25
pinkish	33
crumpled	45
short	67±
Delta	68
crossveinless	68±
bithorax	Between 66 and 70
abnormal	73±
tiny-bristle	98
short-tarsi	98±

FOURTH CHROMOSOME

net	0
antennipedia	31
jaunty	67

(Loci marked "±" are uncertain as to order, as well as to numerical value.)

MAPS OF AUTOSOMES

The second chromosome map shown is more satisfactory than that of the X, since there are more useful characters here. No variations in linkage have been found, indicating that the sequence shown is the usual one throughout the range of the species.

The possible combinations of the three third chromosome characters pink-like, narrow, and roughish have all been studied rather extensively, but no double recessive has yet been obtained. Either all three loci are close

together, or the chromosomes concerned have different sequences. The other mutant genes in this chromosome have not yet been adequately tested.

The fourth chromosome map, like that for the second, shows values that have been obtained repeatedly from crosses to a wide variety of strains. It must represent the sequence usual throughout the range of the species. Preliminary studies show that truncate is approximately 34 units from net; it has not yet been placed with respect to the other two loci shown on the map.

The fifth chromosome has been little studied. Reduced is not favorable for study, since it overlaps wild type frequently, and the complete sterility of abdomen-rotatum makes it also unfavorable. The few tests carried out have not yielded any crossovers between either of these and Fused.

PARALLELISM WITH MUTANT TYPES OF OTHER SPECIES

Comparison of the mutant types here described with those known in *melanogaster* or in *pseudoobscura* (see DONALD 1936, STURTEVANT and TAN 1937) leaves little doubt as to the homologies, which are evidently as follows:

affinis	XL	XR	2	3	4	5
pseudoobscura	XL	XR	2	3	4	5
melanogaster	X	3L	3R	2R	2L	4

In the case of the X this relation was to have been expected, since the X of *melanogaster* seems to include material that is in the X of every species of Drosophila yet studied, while the presence of the sex-ratio gene had previously indicated that the XR of *affinis* agrees with that of *pseudoobscura*. The composition of the fifth chromosome is also the expected one. With respect to the three large autosomes there was no satisfactory basis for a prediction of the constitution of *affinis*. The present data are themselves not sufficient to furnish a final solution here, especially since the position of the centromeres on the maps remains unknown, and the number of parallels is still small. It is still possible that the parallel genes in one or two of the *affinis* autosomes are all in one arm, so that the homology given is not certainly applicable to the entire chromosomes. Nevertheless, the absence of any parallels in disagreement with the suggested correspondence is striking, and may be taken as raising a strong presumption that each autosome of *affinis* contains essentially the same material as does one of those of *pseudoobscura*.

The implications of this result will be discussed below.

THE SUBGENUS SOPHOPHORA

The subgenus Sophophora falls naturally into four species groups, as follows:

1. Dark species; long fine ventral receptacle; long spiral testes; one or

two opaque heavily chitinized areas on the fifth abdominal tergite of the female; skipping larvae; no sex combs; filaments of eggs much expanded at apices. Found in tropical America. *D. saltans* and six undescribed species.

2. Yellow species; long fine ventral receptacle; medium long spiral testes; no opaque areas on tergites; larvae do not skip; no sex-combs; egg filaments much expanded at apices. Found in tropical America. *D. willistoni* and *nebulosa*.

3. Yellow species; rather long ventral receptacle; medium long spiral testes; no opaque areas on tergites; larvae do not skip; sex-combs present. Found in Old World tropics (the first three also in America, where they appear to be introduced). *D. melanogaster, simulans, ananassae, takahashii, montium, auraria, bipectinata*, etc. *D. miki*, from Austria, probably also belongs here.

4. Dark species; short ventral receptacle; testes elliptical or somewhat elongate, scarcely spiral; no opaque areas on tergites; larvae do not skip; sex-combs present; preapical bristles on anterior tibiae unusually long. Found in the north temperate zone (*azteca* and *pseudoobscura* reach Guatemala, but only in the mountainous temperate regions). *D. obscura, subobscura, pseudoobscura, miranda, affinis, algonquin, azteca, athabasca, narragansett*, etc.

At first glance, the relationships between these four groups appear to be those indicated by the sequence given, that is, the second group is intermediate between the first and third, the third between the second and fourth, and the first and fourth most distinct.

The chromosome configurations shown by these species are diverse. *D. willistoni* (group 2) has a V-shaped X, a V-shaped autosome, and a rod-shaped autosome (LANCEFIELD and METZ 1921), as shown in figure 1. *D. saltans* is described as having the *melanogaster* oögonial configuration, which differs only in having a small dot, though the similarity is misleading, since in *melanogaster* the rod, rather than one of the V's, is the X. MR. E. NOVITSKI, in this laboratory, reports that the three *saltans* relatives he has studied have no dot; they may be supposed to have the same complex as *willistoni*. *D. earlei* (from Cuba) was described by METZ (1916b) as having the *willistoni* oögonial configuration; the species is evidently related to the *saltans* group, though it cannot be determined whether it should be placed with the first or the second group here defined (the larvae were not observed to skip, and in the available pinned material it is not possible to be certain whether an opaque area is present on the abdominal tergite). *D. nebulosa* is also listed as having the *melanogaster* oögonial configuration; but examination of the published figures (METZ 1916a) shows that no dots were found. It seems probable that the *willistoni* complex is characteristic of both the first and the second species groups.

Both the third and the fourth groups have a variety of configurations. The *melanogaster* type is found in *simulans*, and is recorded by KIKKAWA and PENG (1938) for *auraria*, *lutea*, *rufa*, and *takahashii*, with *ficusphila* and *suzukii* probably the same but without a positive identification of the X. All these forms apparently belong to the third group of species. (In the cases of *lutea*, *rufa*, *ficusphila*, and *suzukii* this reference is based on the published descriptions, since I have seen no material.) Also in this group

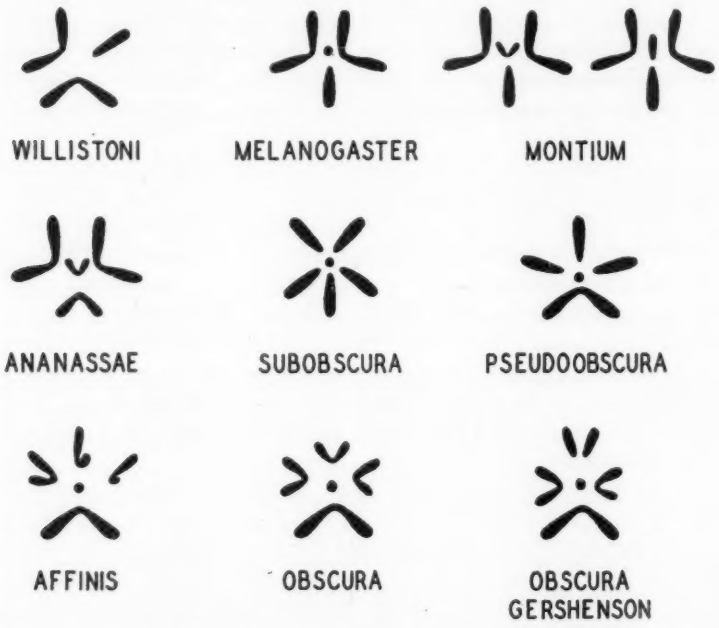


FIGURE 1.—Diagrammatic representation of the chromosome configurations known in the subgenus Sophophora. Haploid groups are shown. In every case the lowermost chromosome is the X; the Y, which is variable even within some of the species, is not shown.

are *montium* and *ananassae*, with the configurations shown in figure 1. *Bipectinata* resembles *ananassae* (KIKKAWA and PENG 1938), though the X has not been identified. KIKKAWA's (1937, 1938) studies show that *ananassae* has the *melanogaster* arrangement with two modifications: the basal portion of X, including much of its heterochromatin, the nucleolus, and the gene bobbed, has become attached to the dot; and the remaining portion of X has acquired a median centromere. The configurations found in *montium* presumably represent the first of these changes (or one similar

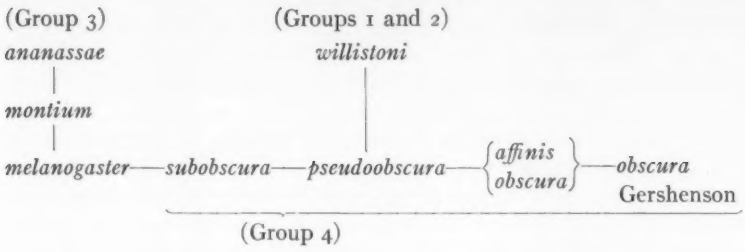
to it—there is no evidence to show how much of X has been transferred to the dot), without the second one. The third group of species is thus characterized by the *melanogaster* configuration or relatively slight modifications of it.

The fourth group of species includes the last five types shown in the figure. Of these, the *affinis* and *obscura* arrangements (found also in *algonquin*, *azteca*, and *athabasca*) are essentially the same, differing only in the relative lengths of the arms of the autosomes. The "*obscura* Gershenson" configuration differs in that one of the autosomes is represented by two rods (see FROLOWA and ASTAUROFF 1929 for the two *obscura* types). *Pseudoobscura* and *miranda* differ from the *affinis* type in that the three J-shaped autosomes have all become rods, and that at least two of them appear to be shorter than in *affinis* or *obscura*. Finally, *subobscura* differs from *pseudoobscura* in having one arm of the X now present as an additional autosome.

The comparison of mutant genes in *melanogaster*, *simulans*, *willistoni*, *pseudoobscura*, and *miranda* shows that in these species the separate arms maintain their identities (see discussion by STURTEVANT and TAN 1937). The X of *melanogaster* and *simulans* remains as one arm of X in the other three species, and the other arm of X in these three species is the equivalent of 3L of *melanogaster* or *simulans*. The present account confirms the suggestion of STURTEVANT and DOBZHANSKY (1936) that the X of *affinis* has the same composition as that of *pseudoobscura*. The work of KIKKAWA (1937, 1938) shows that the X of *ananassae* is the same as that of *melanogaster*, except that the bobbed end has been removed and attached to the dot, while the centromere of the remaining portion has become median. The composition of the X is thus fairly clear throughout the subgenus.

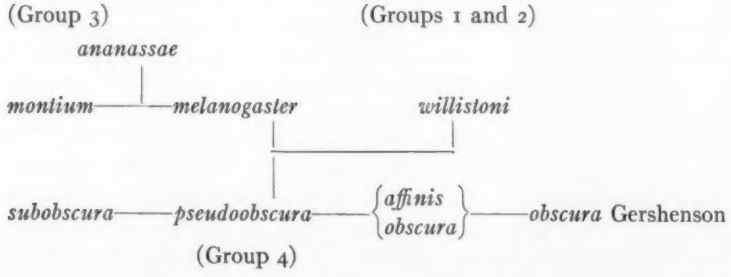
With respect to the autosomes there are two chief problems: (1) where is the dot material in the *saltans* and *willistoni* configuration; (2) what is the content of the J-shaped autosomes of *affinis* and *obscura*? The first question cannot be answered from the available evidence; the second is answered in part by the data presented in this paper. It is probable that each autosome of *affinis* corresponds largely or entirely to one autosome of *pseudoobscura* or to one autosomal arm of *melanogaster*. The changes involved have, then, apparently been merely in the position of the centromeres and associated heterochromatin. This interpretation must be accepted with caution, however, as pointed out above.

The historical interpretation suggested by these configurations is indicated in the accompanying diagram. It should be emphasized that the names in the diagram refer to chromosome configurations as shown in figure 1, not to the species themselves.



Analysis of the characters of the animals by the methods suggested previously (STURTEVANT 1939) is in agreement with this arrangement except that groups 1 and 2 are to be related to group 3 rather than to group 4, an interpretation that may be reconciled with the cytological and genetic data if group 3 and *subobscura* are supposed to represent independent origins of a rod-shaped X. It may be noted that *subobscura* does not appear to be as similar to the members of group 3 (in characters other than chromosomes) as do the members of the *affinis* sub-group. The closest intergroup resemblances here are betweeen the members of the *affinis* and *melanogaster* sub-groups.

The diagram may be modified as shown below, in order to produce a compromise between the two sets of characters—chromosomal and non-cytological. This scheme is, naturally, to be taken only as a working model. The study of additional species, especially from the eastern Palaearctic region, is perhaps the greatest present desideratum for its improvement. (No member of group 4 has been seen from the area between Moscow and Alaska.)



If the diagram is accepted as giving an approximate picture of the phylogeny of the subgenus, the next question that arises is—what is the point of origin? This question may be approached in several ways. One method is through comparative cytology. Of the various chromosome configurations shown, the ones that are known outside of the subgenus are:

melanogaster type—in *Chymomyza amoena, Scaptomyza graminum*, and (with doubt as to which chromosome is the X) in *Mycodrosophila dimidiata, Chymomyza procnemis, Chaetopsis fulvifrons*, and the following species of Drosophila (all of uncertain subgeneric reference, but probably neither Sophophora nor subgenus Drosophila): *bromeliae, florae, coracina. Quinaria* and *robusta*, of the subgenus Drosophila, have been referred here, but *robusta* is certainly different, and the published figures for *quinaria* show no dot and do not indicate which is X.

subobscura type—in *ramsdeni, virilis, similis, cardini, mulleri, phalerata, transversa*, all belonging to the subgenus Drosophila. Essentially the same arrangement, with differences only in amount and distribution of heterochromatin, occurs also in the following members of the subgenus Drosophila: *funebris, histrio, hydei, repleta.*

pseudoobscura type—in *virilis americana* (HUGHES 1939), where it is evidently a secondary derivative of the *subobscura* type.

affinis type—in *duncani* (DOBZHANSKY, unpublished), which is the type of an unnamed subgenus (referred by STURTEVANT (1939) to Dasydrosophila Duda, but not in reasonable agreement with the type of that subgenus).

This comparison thus suggests that species-group 3 is related to Chymomyza and Scaptomyza and less certainly to Chaetopsis (of the very remote family Ulidiidae), Mycodrosophila, and two little-understood subgenera of Drosophila; and that species-group 4 is related to the subgenus Drosophila and to *duncani*. There is no indication that groups 1 and 2 are connected directly with any other form that has been studied.

The method of comparing resemblances in many different characters (STURTEVANT 1939) is in general agreement with this. The closest resemblance of any species of Sophophora with any of the other species examined is that between the *affinis* forms and an undescribed member of the subgenus Drosophila from the mountains of California. It should be pointed out that none of the forms listed above as having the *melanogaster* group is included in these latter comparisons. An undescribed American species that is close to *coracina* is included; it does not have the *melanogaster* chromosome group, and does not appear to be closely related to *duncani* or to any of the species of Sophophora or Drosophila that have been examined.

Another method of approach is through examination of the characteristics of each species group, to determine which seem most likely to be special and recently arisen peculiarities. In this category of characters are to be placed the opaque areas on the tergites (group 1), and sex-combs

(groups 3 and 4). It is also rather probable that relatively short ventral receptacles and testes (group 4, and group 3 to a lesser degree) are primitive. This method may be taken as agreeing with the others in indicating that group 1 is not the point of origin for Sophophora, and in suggesting group 4 as the more primitive—though group 2 is also to be considered as a possibility.

The most obvious inter-subgeneric relationship is that between group 4 and the subgenus Drosophila. It should be pointed out that Drosophila is a more complex subgenus than Sophophora, including more species groups, whose interrelationships and geographical distribution are more difficult to describe. It seems likely, therefore, that Drosophila is at least as old as Sophophora—again suggesting group 4 as the most ancient one in Sophophora.

SUMMARY

1. Forty-seven mutant types of *Drosophila affinis* are described, dependent on mutant genes in 40 different loci. Of these, 13 are in the X chromosome, 15 in the second, 5 in the third, 4 in the fourth, and 3 in the fifth.

2. Twenty-eight of these types resemble ones previously known in *D. melanogaster* or *D. pseudoobscura*. Their distribution among the chromosomes indicates the following homologies:

affinis	XL	XR	2	3	4	5
pseudoobscura	XL	XR	2	3	4	5
melanogaster	X	3L	3R	2R	2L	4

3. There are no probable parallels in disagreement with these homologies.

4. The subgenus Sophophora falls into four species-groups. A discussion of the morphology and cytology of the available representatives of each of these groups leads to a tentative phylogeny. The species-group that includes *pseudoobscura* and *affinis* is perhaps nearer the ancestral type than are the other three. This leads to the supposition that the *melanogaster* chromosome group was derived from the *pseudoobscura-affinis* arrangement.

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AN ANALYSIS OF THE BRISTLE FORMING REACTIONS IN DROSOPHILA MELANOGASTER SCUTE-1

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Received March 1, 1940

INTRODUCTION

THAT portion of development during which a change in temperature produces a differential effect on the expression of some character is the temperature-effective period (TEP) for that character. Such periods have been determined for several characters of *D. melanogaster*, HOGE (1915), KRAFKA (1924), DRIVER (1931), E. DRIVER (1925, 1931), LUCE (1931), PLUNKETT (1926), STANLEY (1932), and HARNLY (1932, 1936). Some of these workers have attempted to explain the mechanism of the temperature effect by the form of the curves obtained when the values of the measured character are plotted against the time of transfer. It is significant that the form of these curves, in all cases, appears to be sigmoid, resembling to some extent the probability integral curve. CHILD (1935b) and MARGOLIS (1935) have suggested that the form of these curves for the bristles of scute-1 and the facets of Bar eye, respectively, may be determined chiefly by the distribution in time of the TEP in the individual flies. In other words larvae hatching from eggs laid at the same time do not all develop at the same rate and hence enter their individual TEP's at different times. The TEP of the population (P) is the summation of two factors 1) the variation (V) among the larvae in time of entering the TEP and 2) the duration (D) of the TEP in the individual flies, $P = V + D$. It was shown that V could be represented by a normal frequency distribution.

In this paper the data are analyzed further to obtain the curves and equations of the developmental process leading to bristle number determination in scute-1. Using these equations and the curve of the probability integral it is further possible to calculate the P curve.

EXPERIMENTAL

The culture methods and the tests for homogeneity of the stock were reported previously (CHILD 1935a). One and two hour egg-laying periods were used. Groups of developing flies were transferred from 28° to 20° during P and larvae were removed to agar slants at two-hour intervals as they pupated. The mean bristle numbers of these pupating groups were recorded separately at eclosion. A number of such experiments were conducted, the transfers being made at various times during P. Table 1 shows the results on the ocellar (oc) and anterior notopleural (anp) bristles for the females. The bristle numbers are expressed as mean per half fly, for

TABLE 1

♀ ♀

Relation between bristle number and time at which flies pupate after being transferred from 28° to 20° during P at time indicated in column heading.

82 HOURS				86 HOURS				87.5 HOURS			
t.	NO.	MOC	MANP	t.	NO.	MOC	MANP	t.	NO.	MOC	MANP
96	1	1.0	0.5	88	1	1.0	0	85.5	1	1.0	0.5
98	1	0.5	0.5	90	0			87.5	2	0.75	0.75
100	*2	0	1.0	92	3	1.0	0.83	89.5	4	0.62	0.62
102	1	0	0	94	3	0.66	1.0	91.5	0		
104	13	0	0.04	96	2	0.75	1.0	93.5	8	0.75	0.44
106	17	0.06	0.06	98	*8	0.19	0.75	95.5	24	0.62	0.60
108	24	0.02	0	100	32	0.14	0.42	97.5	*54	0.36	0.55
110	37	0	0.02	102	32	0.03	0.43	99.5	80	0.32	0.46
112	30	0	0.02	104	45	0.11	0.21	101.5	73	0.16	0.33
114	44	0	0	106	7	0	0.14	103.5	74	0.07	0.26
116	30	0	0	108	9	0	0	105.5	79	0.05	0.22
118	25	0	0	110	7	0	0	107.5	60	0	0.07
120	13	0	0	112	5	0	0	109.5	53	0	0.04
122	9	0	0	114	7	0	0	111.5	26	0	0
124	9	0	0	116	4	0	0	113.5	16	0	0
126	6	0	0	118	3	0	0	115.5	14	0	0
128	9	0	0								
130	6	0	0								

96 HOURS				93 HOURS				88.5 HOURS			
86	3	0.66	0.83	85	1	1.0	1.0	86.5			
88	5	0.7	0.5	87	3	0.83	0.5	88.5	5	1.0	0.6
90	8	0.5	0.44	89	4	0.63	0.63	90.5	2	0.75	1.0
92	16	0.63	0.61	91	8	0.56	0.56	92.5	7	0.57	0.5
94	32	0.61	0.71	93	12	0.83	0.46	94.5	17	0.56	0.91
96	19	0.58	0.65	95	18	0.67	0.69	96.5	*23	0.46	0.61
98	10	0.9	0.65	97	35	0.59	0.56	98.5	22	0.16	0.63
100	17	0.50	0.61	99	20	0.59	0.63	100.5	17	0.29	0.41
102	16	0.66	0.59	101	*39	0.36	0.49	102.5	9	0.22	0.55
104	*14	0.25	0.65	103	33	0.29	0.5	104.5	5	0.10	0.4
106	8	0.37	0.69	105	33	0.17	0.3	106.5	13	0.00	0.38
108	13	0.15	0.65	107	23	0.17	0.28	108.5	5	0.0	0.10
110	8	0.19	0.31	109	23	0.09	0.11	110.5	2	0	0
112	1		0.5	111	9	0.11	0.16	112.5	2	0	0
114	2		0.5	113	14	0	0.04	114.5	2	0	0
116	1		0	115	0						
				117	0						
				119	22	0	0				

example, if among 100 flies 30 ocellar bristles were found the Moc=0.15. Similar results were obtained for males.

The flies pupating in the successive two-hour intervals from each transfer period may be classified into three groups on the basis of mean bristle

number: 1) those having passed through D at the time of transfer; 2 (those still in D at the time of transfer; 3) those having not as yet entered D at the time of transfer. Groups 1 and 3 measure the end and the beginning of D, respectively. The mean bristle numbers of group 2 when plotted against time of pupation measures the course of the reaction during D.

TABLE 2

Larvae transferred from 28° to 20°. Pupae removed at two hour intervals and mean bristle number per half fly determined for each group at eclosion.

	NO. ♀♀ FLIES PUPATED			NO. ♂♂ FLIES PUPATED		
		MOC.	MANP.		MOC.	MANP.
28° control	760	0.60	0.46	685	0.41	0.037
	3	0.66	0.83			
	6	0.75	0.58			
	11	0.64	0.50	81	0.44	0.037
	21	0.66	0.48	184	0.47	0.038
	43	0.63	0.63	143	0.41	0.051
	40	0.71	0.51	86	0.55	0.041
	33	0.75	0.47	158	0.54	0.050
	71	0.59	0.53	186	0.44	0.038
	80	0.62	0.56	168	0.23	0.044
	140	0.35	0.55	135	0.13	0.037
	176	0.26	0.49	139	0.050	0.043
	181	0.14	0.36	88	0.011	0.023
	176	0.090	0.30	51	0	0.010
	139	0.051	0.18	34	0	0.014
	130	0.008	0.092	23	0	0
	110	0	0.032	17	0	0
	77	0	0	21	0	0
	55	0	0	14	0	0
	67	0	0			
	16	0	0			
	9	0	0			
	9	0	0			
	6	0	0			
	9	0	0			
	6	0	0			
20° Control	605	0	0	619	0	0

Unfortunately there are not enough flies in group 2 from each separate transfer period and it is therefore necessary to summate all the data. The data could be added together by making the time of transfer the starting time for each transfer period. This was not done since the results show that the TEP's in the individual flies do not begin at the same time in relation to the time of pupation in the various transfer groups. It is not possible, therefore, to use time of transfer as the zero time point in summating the data. These data were added together after aligning the pupat-

ing periods in which the change in temperature produced a noticeable effect on the bristle number. These periods are indicated by an asterisk. In other words, the beginning of D was determined for each group separately and then all the data were added together on this basis. The results are given in table 2. The control values for flies raised at 20° and 28° for their whole developmental periods are also given. The two hour periods between each group are at 20°. To convert this to time at 28° it is necessary to use the factor 94.1/185.4 and 89.6/178.6 for the females and males respectively. These figures are the durations in hours of the egg-larval periods at 28° and 20°.

ANALYSIS OF THE DATA

Before attempting an analysis of the data it is necessary to consider a fundamental assumption which must be made concerning the "all or none" behavior of a particular bristle in one fly and its relation to the mean bristle number of that bristle in the whole population. It is to be assumed that the mean bristle number of a particular bristle in a population of flies, uniform as to genetic and environmental factors, is proportional to the amount of bristle substance or precursor (for that bristle) present in that population at the time of bristle determination. This assumption implies that 1) there is a distribution in the amount of bristle substance among the flies, 2) a bristle will be present if the concentration of bristle substance exceeds a 'threshold' concentration and absent if below this threshold, 3) the value of this threshold and the nature of the distribution are such as to allow the mean of the distribution to be proportional to the mean bristle number. At the present time I can see no clear mathematical proof for this fundamental assumption. The fact that the data treated in this manner yield conclusions from which other results may be predicted makes the assumption necessary. PLUNKETT (1926) has also utilized this assumption in his analysis of the Dichaete gene and has considered its implications in greater detail than has been done here.

It has been shown (CHILD 1935a) that this scute-1 stock does not develop any ocellar or anterior notopleural bristles below 22°. It may be assumed therefore that below this temperature the reaction producing "bristle substance" goes on at a very slow rate and may be neglected. The data, on the basis of these assumptions describe the formation of "bristle substance" or precursor at 28°.

The beginning and the end of the reactions are taken as the periods in which the pupating groups give bristle numbers equal to the controls at 20° and 28° respectively. Only the oc and anp bristles are under consideration since there are few data on the other bristles.

Unsuccessful attempts were made to fit these data into a uni- or bimolecular reaction system. The mean bristle numbers rise rapidly indicat-

ing a logarithmic or exponential system. Plotting log time versus log bristle number (figure 1) gives reasonably good results. The straight lines were determined by the method of least squares. The anp male line is not satisfactory since the first point is based on only 34 flies and is not very significant. The other three points describe a line which would be more in harmony with the other bristle data. The equation for these log-log plots, log bristle number = k log time + constant, is essentially the type of equa-

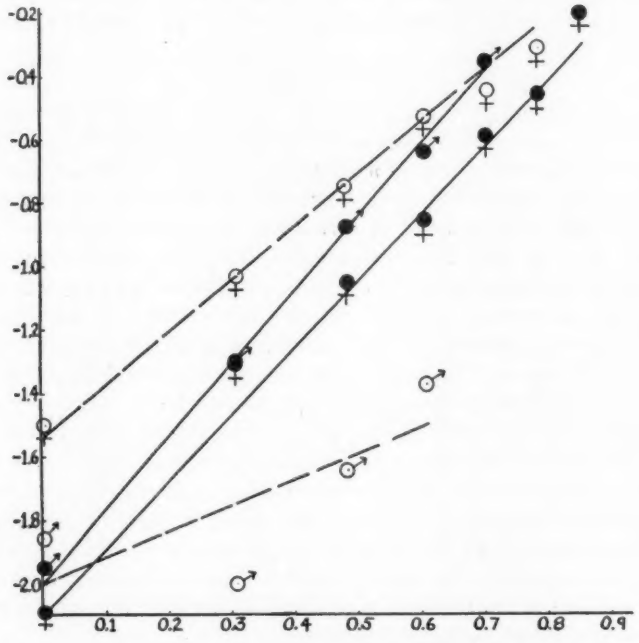

FIGURE 1.—The bristle reaction, log mean bristle number = k log time + constant. Ordinates, log mean bristle number; abscissae, log time o = anp, ● = oc.

tion from which HUXLEY (1932) derives his law of heterogonic growth. The differential form of this equation $db/dt = k(b/t)$ implies that the rate of formation of bristle substance at time t is proportional to the average rate up to that time. This equation has been used by a number of investigators to describe organic growth (see NEEDHAM 1931). GLASER (1938) using another form of the equation $dw/dt = k(dt/2t+1)$ applied it with considerable success to many categories of growth data (w = weight of the organism or some part of it). It may be mentioned here that since the log oc and log anp are both related to log t, plotting log oc versus log anp

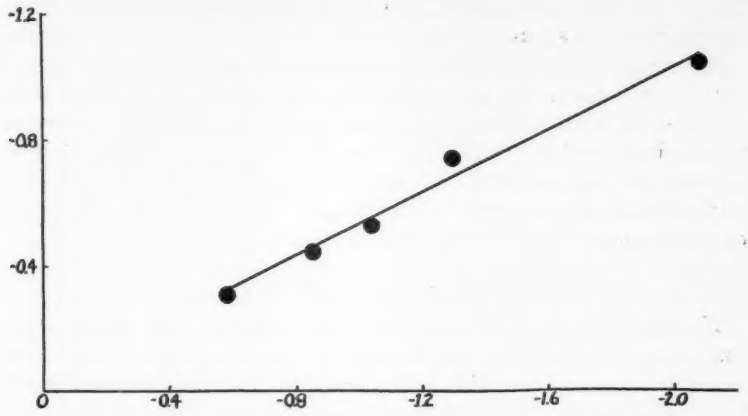

FIGURE 2.—The heterogonic relation between the bristles. Ordinates, log oc, abscissae, log anp.

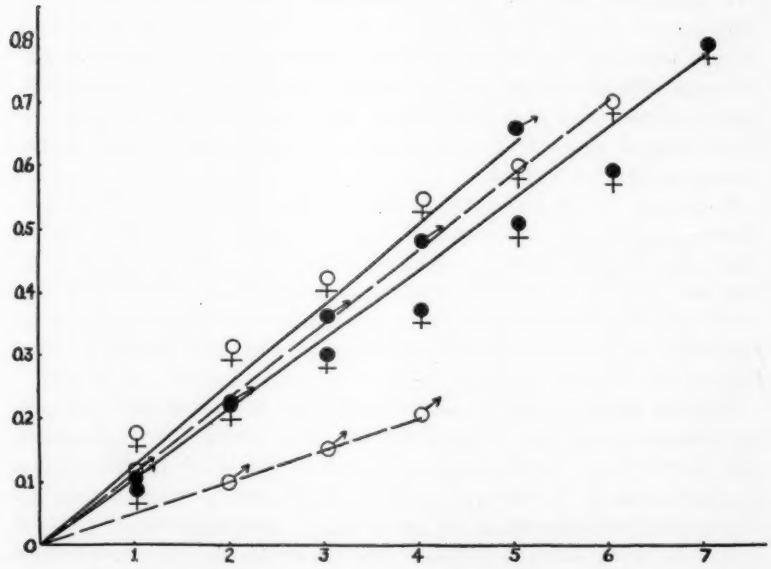

FIGURE 3.—The bristle reaction, mean bristle number$^{1/2}$=constant×time. Ordinates, bristle number$^{1/2}$; abscissae, time ○=anp, ●=oc.

should give a straight line. Figure 2 shows that this is the case. This establishes a heterogonic relationship between the oc and anp bristles. NEEL

(1940) found that bristle number and body weight in his experiments are also related in this manner.

Too much significance, however, should not be attached to these equations since they do not adequately describe the kinetics of the bristle reaction and other empirical equations would do as well. The expression mean bristle number$^{1/2}$ = constant × time which is simpler than the log-log equation also appears to fit the data (figure 3). The slopes of these lines were calculated by dividing the durations of the reactions by the square root of the mean bristle numbers of the controls at 28°.

ANALYSIS OF BRISTLE REACTIONS

The lines show that the "rates of bristle formation" are not the same. The duration of the oc reaction is about one hour longer than that of the anp in both sexes. The reactions are about two hours shorter in the males than in the females. The oc reaction also starts earlier than the anp as can be seen from the table. Thus the anp male reaction lasts about four hours, the oc male five hours, anp female six hours and the oc female seven hours. The characters, mean bristle numbers are, on this basis, the results of differences in rates and durations of bristle reactions for the oc and anp bristles and in the two sexes. This type of generalization regarding characters has been made by many investigators with few definite measurements on the rates and durations of the developmental reactions (see GOLDSCHMIDT 1938). The analysis of the bristle numbers gives definite values to these parameters.

The analysis does not show, however, what the nature of the bristle forming reaction may be. Since at this larval period there are still no visible signs of bristles (ROBERTSON 1936), the reactions at this period are probably not on the morphological level but must be on a chemo-embryological level. That is, actual bristle formation is not involved. The change in the amount of bristle precursor is being affected during this period of development.

The equations may give a clue to the kinetics of the bristle precursor reactions. The differential form of $b^{1/2} = ct$ is $db/dt = 2cb^{1/2}$. The other equation $db/dt = k(b/t)$ is identical with $db/dt = ak(b)^{1-1/k}$ in which a is a constant obtained by integration. These equations are similar in form to the types of equations found in the study of heterogeneous chemical reactions involving adsorption that is, $dp/dt = kp^n$ in which p is the pressure of a gas being adsorbed before it can react. The constant n is usually between 0 (zero order) and one (unimolecular) and in many instances is equal to $\frac{1}{2}$. Some of the reactions in which $n = \frac{1}{2}$ have been interpreted as diffusions.

On the basis of this analogy a number of mechanisms may be suggested

for the bristle substance reaction. The following analysis indicates one possible type of mechanism. In the early development of the larva, bristle substances are being synthesized in reactions which are not differentially affected by the presence of the scute-1 gene or its byproducts, that is, these reactions are the same in all respects as the reactions in the wild-type fly. As development proceeds to a certain time and stage, the processes which are affected by the presence of scute-1 (or its wild-type allele) begin, that is, these reactions are not the same in all respects as their homologous reactions in the wild-type fly. The time when the reactions begin, is the beginning of D. These "scute-1 controlled" processes may be an adsorption or diffusion of substances from a site of synthesis to a place at which future essential bristle forming reactions will occur. These diffusion or adsorption reactions cease at a time corresponding to the end of D. For the remainder of development or at least up to the time at which the presence or absence of a bristle is finally determined, the bristle reactions are the same in both wild-type and scute fly. These reactions, however, are affected by the amount of substrate received during the scute-1 reaction so that the bristle number of the adult scute-1 fly has been determined essentially by the scute controlled reaction.

It is common knowledge that the bristle numbers of the wild type are not affected by temperature changes (within this normal temperature range) during development. The results of IVES (1939) and of CHILD, BLANC and PLOUGH (1940) show, however, that bristle reactions take place in the wild-type fly during the period comparable to D in scute-1. If these diffusion or adsorption reactions do occur in the wild-type fly they must have higher velocity constants or longer durations to bring about a full bristle number in all flies. The temperature coefficients of these wild-type bristle reactions during D must also be in harmony with that of other developmental reactions so that the rate or durations of the bristle reactions may result in full bristle numbers at all temperatures. In temperature coefficient these reactions in the scute-1 fly, however, are not in step with the rest of the developmental processes so that scute-1 flies raised at different temperatures have rates of D which do not bear the same relations to the rates of other developmental processes at all temperatures. This differential effect of temperature in the scute-1 fly results in a change in bristle number at different temperatures. It further permits of the use of temperature to measure the rate, duration and temperature coefficient of the bristle reaction which differentiates between the presence of scute-1 or its wild-type allele in a developing fly. It is evident that the developmental processes leading to bristle formation are made less efficient by the substitution of scute-1 for the wild-type allele.

CALCULATION OF THE P CURVE FROM V AND D

As mentioned previously, the variation in time at which the larvae enter their temperature effective periods follows the probability integral curve. At 28° the practical limits of this curve ($V \pm 3\sigma$) are approximately 18 hours. Taking the oc bristles in the female as a type case, the P curve may be calculated from V and curve of D. From a probability table or curve it is seen that in a population transferred 1σ or three hours after the beginning of P 97.7 percent of the larvae have not entered their TEP, 1.9 percent have been in it for 0.5 hours and .4 percent for 2 hours. From the equations of D the bristle numbers of these groups may be determined. The average of this population is 0.0003. In a population transferred at

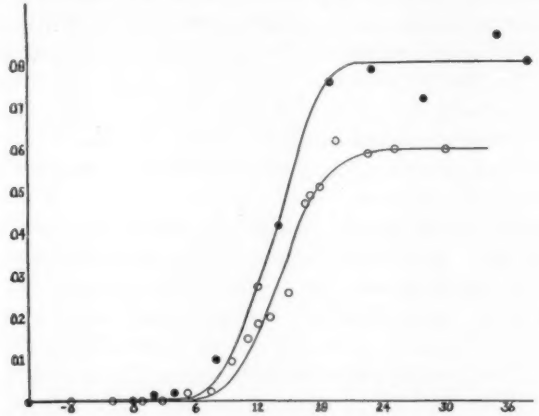

FIGURE 4.—Calculated P curves. Experimental points obtained by measuring the bristle numbers of populations of flies transferred from T_1 to T_2 during development. Ordinates, mean oc ♀ ♀ bristle numbers: abscissae, time in hours from the beginning of P. ○=$T_1$28°, $T_2$28°; ●=$T_1$29.7°, $T_2$22.0°.

2σ or six hours 84 percent have not entered, the periods for the remaining 16 percent obtained from V and their bristles numbers from D. The average is taken and this gives the six hour point on the P curve. In a similar manner points are obtained for 9, 12, 15 and 18 hours. Figure 4 shows the smooth curve obtained from this calculation. When the experimental values obtained from many earlier experiments (CHILD 1935b) are superimposed on this line as in figure 4 it can be seen that there is reasonable agreement. In a similar manner the P curves for the other bristles have been calculated. It follows from these calculations that P can be obtained from V+D and that interpretations of the nature of the developmental processes leading to character formation cannot be made from curves of P. If the TEP is to be studied the D curve must be determined.

CALCULATION OF P AND D AT OTHER TEMPERATURES

To calculate the curves of D and P at other temperatures it is necessary to assume that the durations of D and V are proportional to the time of development at these temperatures. This is not unreasonable since it was shown (CHILD 1935b) that the duration of P is approximately proportional to the time of development. The mean bristle number and the time of development have been previously reported (CHILD 1935a, b). Substituting these values in the equations $\log b = k \log t + \log a$ (assume constant a) and $b^{1/2} = c^t$, the equations for the curves of D at these temperatures may

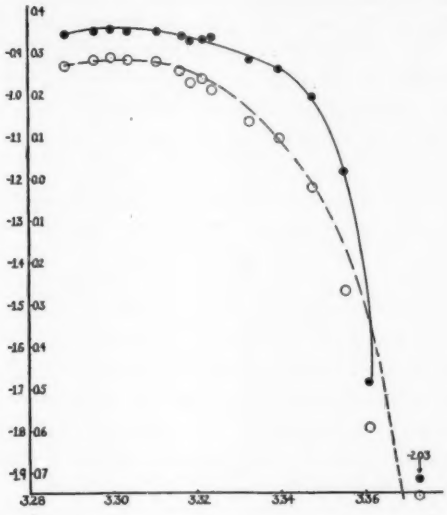

FIGURE 5.—Relation between temperature and velocity constants of the bristle reaction. Ordinates, inner scale (and solid line) log k from log-log equation, outer scale (and broken line) log c from half-power equation; abscissae, reciprocal absolute temperature $\times 10^{-3}$.

be obtained. Since k and c represent "velocity constants," it was of interest to determine whether they were related to temperature as in the Arrhenius equation $d \log k/dT = u/RT^2$. In plotting log c and log k against $1/T$ (figure 5) straight lines are not obtained, that is, u is not constant. This is not surprising, however, since very few biological reactions have constant temperature coefficients. These curves, however, give the values of k and c at all temperatures. By substituting these values into the D equations the course of a bristle reaction may be described at all temperatures. Using the curves of V and these curves D as in previous calculation for P at 28°, the curves of P at all temperatures may be calculated. Figure

4 shows the curve of P for transfers between 29.7° and 22°. The experimental points are too few to make a reasonable comparison with the calculated curve.

SUMMARY

The sigmoid curves (P) which are usually obtained in the determination of temperature-effective periods are the sum of at least two curves resulting from, 1) the variation (V) from fly to fly in the time at which their temperature-effective periods begin and, 2) the rate and duration (D) of the affected reaction in the individual flies. The separate curves of V and D have been obtained for scute-1 by an elaboration of the usual methods for determining temperature-effective periods The results indicate that in a population of developing flies obtained from an egg-laying period of 1–2 hours the variations in the time at which the larvae enter their sensitive periods follow a normal distribution curve.

The curves showing the rates and durations of the various bristle forming reactions are also obtained from these data. An analysis of the D curves indicates that they fit the equation $\log b = k \log t + \text{constant}$ where b = mean bristle number per half fly, t = time, and k is a constant. The constants and the durations of the reactions are not the same for the various bristles. The data also fit other similar equations including a simple form $b^{1/2} = ct$. These equations are similar to the equations found in heterogeneous chemical reactions. The kinetics of the scute-1 reaction are discussed from this point of view. Using the curves of D and V it is possible to calculate the P curves. Interpretations of the mechanism of developmental processes therefore cannot be made from curves measuring the temperature effective period of the population but require a study of the reactions in the individual fly.

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GAMETIC LETHALS ON THE FOURTH CHROMOSOME OF MAIZE*

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PLANTS of maize, heterozygous for *su*, have been observed that produced no *su* seeds when selfed or pollinated by *su* pollen, but whose pollen when applied to *su* ears produced in excess of 96 percent *su* seeds (figure 2, H, I). This anomalous condition is caused by two gametic lethals, small pollen-1, (*sp*), and lethal ovule-1 (*lo*).[1] Preliminary reports on the inheritance of these two factors have been published, (MANGELSDORF 1931, 1932, SINGLETON 1932). Both *sp* and *lo* are closely linked with *su*(*sp su* = 5 percent recombinations; *su lo* = 2.2 percent) and give distorted ratios of *Su*:*su* because of this close linkage and the fact that one gene is lethal to the male, the other to the female gametes. Pollen grains of the composition *sp* are smaller than normal (figure 5, C, D, and E) and in most cases do not function in competition with normal grains. Ovules carrying *lo* usually abort before fertilization and are rarely capable of being fertilized. The ears referred to above were of the composition *sp*++/+*su lo*. The only functional ovules were of the composition *Lo* which were mostly *Su*, in a few cases all *Su*, when the crossing over may have been reduced or due to random sampling an occasional ear was all *Su*. The functional pollen was *Sp* in constitution and was *su* with the exception of recombinations.

ORIGIN OF *sp* AND *lo*

The gametic lethal *sp* was discovered by the junior author while on the staff of the Connecticut Experiment Station in 1924. This factor must have arisen as a mutation in a Leaming strain, Connecticut 112–8, that had been inbred four generations. It is not possible to say in what generation the *sp* factor arose although it is practically certain to have occurred after the first generation, since the original ear selfed once showed no missing kernels, a characteristic of *sp*/+ ears. This condition, when it first appeared, was in the repulsion phase, *sp* linked with *Su*, which accounts for the high percentage of surgary seeds when selfed or backcrossed either way.

The *lo* factor arose in a stock segregating for *sp* and *su*. It must have arisen as a mutation on the homologue of the chromosome carrying *sp*,

* The cost of the accompanying half-tone illustrations has been borne by the Galton and Mendel Memorial Fund.

[1] Factor symbols not accompanied by numerals represent either the first or the only gene with that literal symbol; that is, *sp* = sp_1, etc.

since *lo* was linked with *su* when first observed while *sp* was linked with *Su*. The most probable explanation of the origin of *lo* is that its occurrence in a stock segregating for *sp* was purely a coincidence although there is a possibility of a causal relationship. The first *lo*/+ ear had 11.1 percent of *su* seeds. The two preceding generations had 53 and 64 percents respectively, showing presence of the *sp* factor but absence of the *lo* factor. All studies of the *lo* condition were made by the senior author in Connecticut. The inheritance of this factor was complicated at first because the stock was also segregating *sp* and gave the unusual results described above. However the two factors have been separated and each studied independently and in combination.

INHERITANCE OF *sp* AND *lo*

Disturbance of su ratios

Both the factors *sp* and *lo* were discovered because of their close linkage to *su* and the inheritance of each has been studied largely through its disturbance of the *su* ratio in selfed and backcrossed ears. The *sp* condition was originally termed "high sugary" since it was in the repulsion phase, *sp*+/+*su*, and gave a high percentage of *su* kernels (figure 1).

The other gametic lethal, *lo*, was originally called "low sugary" because the coupling phase, *lo su*/++, the one first observed, gave a very low percentage of *su* kernels. The factor *sp*, produces extremely low sugary ratios when in the coupling phase *sp su*/++, while the original "low sugary," *lo*, produces very high sugary ratios in the repulsion phase *lo*+/+*su* (figure 2).

The disturbance of the *su* ratio in stocks heterozygous for *lo* or *sp* can perhaps best be understood by presenting in tabular form the functional male and female gametes. This is presented in table 1.

TABLE 1

Functional gametes in plants segregating for sp, lo, or sp and lo not counting double-crossovers or survival of sp ♂ gametes or lo ♀ gametes.

GENOTYPE	FUNCTIONAL GAMETES ♀	♂
sp +/+ *su*	*sp* +, + *su* *sp su*, ++	+ *su*, ++
lo su/++	++ + *su*	*lo su* + + *lo* +, + *su*
sp su +/++ *lo*	*sp su* + + *su* + + + +	+ + *lo* + *su* + + + +

Linkage relations of sp with su

Since in preliminary trials it was found that *sp* functions quite rarely (about one percent of the progeny plants were *Sp sp;* MANGELSDORF 1932) in competition with normal pollen, the backcross of *su su* ears by pollen from *sp*/+ plants has been used to give the crossover percent directly. Table 2 gives the total counts of all self pollinations or backcrosses in Connecticut and Texas.

TABLE 2

Percentages of su seeds obtained for selfed ears, and backcrosses to su for stocks segregating for sp, lo, or sp and lo.

GENOTYPE	SELF		TIMES *su*		POLLEN ON *su*		EXPECTED SELF RATIO (5×7)	DIFFERENCE (3–8)
	TOTAL	% *su*	TOTAL	% *su*	TOTAL	% *su*		
1	2	3	4	5	6	7	8	9
sp +/+ *su*	143M	60.8	36M	61.9	158M	93.9	58.1	+2.7
sp su/++	7M	3.2	6M	37.6	41M	9.7	3.6	− .4
lo su/++	31M	1.8	54M	2.4	18M	51.3	1.2	+ .6
lo +/+ *su*	8M	47.1	27M	95.2	34M	48.5	46.2	+ .9
sp ++/+ *su lo*	7M	5.2	1M	8.5	14M	91.6	7.8	−2.6
++ *lo*/*sp su* +	2M	4.8			15M	26.5*		

* Only 28.6 percent of these are recombinations (true C.O. value = 7.6) remainder represent functional *sp* male gametes.

The percent of *Su* seeds, 6.1, in the backcross *su*×*sp*+/+*su* is the maximum average percentage of recombination between *sp* and *Su* in the repulsion phase, and represents the actual crossover value if the amount of functioning of *sp* pollen is negligible.

Functional *sp* pollen

Several cases of supposedly high crossover ratios have been found during the course of this investigation. In 1926 and 1927 the percents were 10.1 and 19.3 based on totals of five and one thousand seeds respectively. Also in 1932 several progenies were found that gave rather high recombination values. These could be explained either by an actual increase in the crossing over or by functioning of the linkage class of *sp Su* pollen grains. To test these two alternatives, some of the *Su* crossover seeds produced by the pollination *su*×*sp*+/+*su* were grown. Pollen was examined to determine whether normal plants or plants segregating *sp* had been obtained. The results of all such examinations are given in table 3. Certain facts are obvious in this table. Of a total of 2294 plants examined, 15.3 percent resulted from functioning *sp* pollen grains. This is considerably greater than the one percent previously reported. It may be argued that the value of 15.3 percent is too high since we were testing, in some cases, progenies

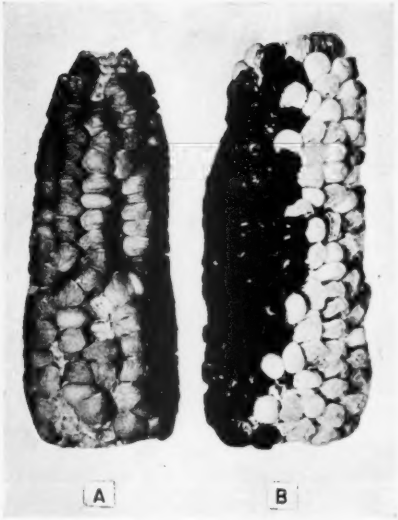

FIGURE 1.—Self pollination and backcross to *su* of *sp*+/+*su* plant. A -*su* times pollen from heterozygous plant; very small percent of *Su* seeds, the crossover class (11.6 percent). B shows a dual pollination, colorless seeds representing self pollinations, the black seeds being pollinated by *ACRPr su*. In making this pollination silks are divided approximately equally and different pollen applied to each half of the silks. Selfed seeds=58.0 percent *su*, ×*su*=66.1 percent *su*.

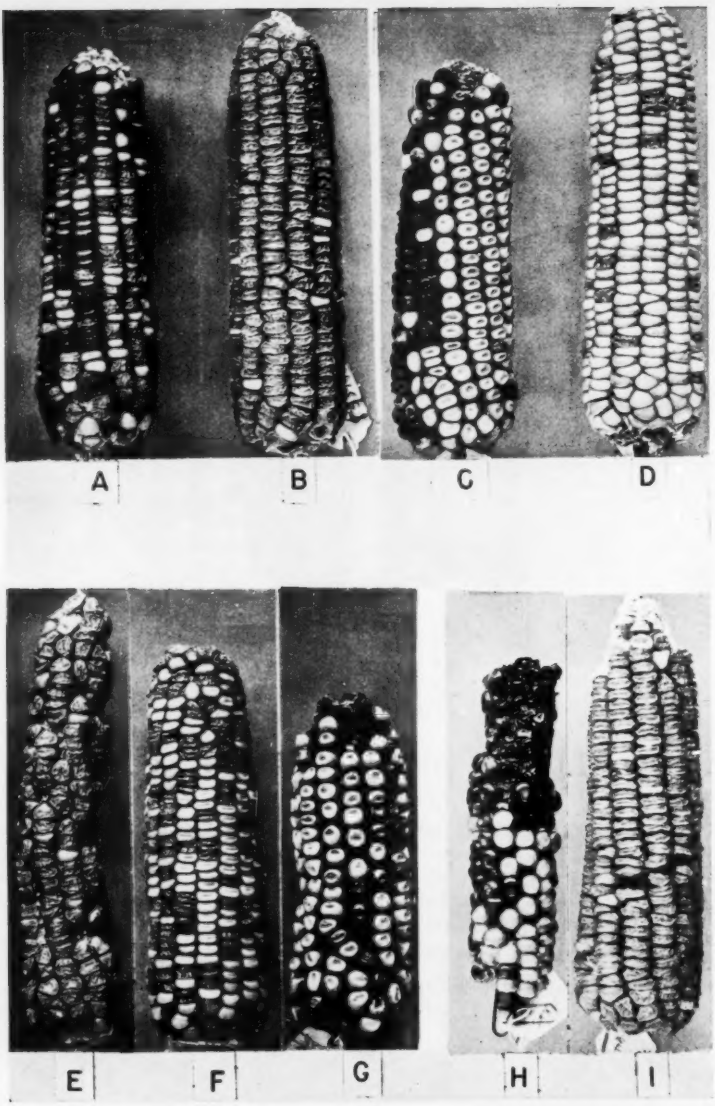

FIGURE 2.—A=ear from *sp*+/+*su* plant selfed (58.4 percent *su*), and crossed by *ACRPr su* (56.5 percent *su*); B=pollen from A put on *su* ear (97.1 percent *su*); C=*sp su*/++ ear selfed (2.2 percent *su*) and crossed by *ACRPr su* (1.6 percent *su*); D=pollen of C on *su* (6.1 percent *su*); E=*lo*+/+*su* ear crossed by *su* (95.2 percent *su*); F=pollen of E on *su* (49.9 percent *su*); G=*lo su*/++ ear selfed (0 *su*, 180 seeds) and crossed by *su* (0 *su*, 20 seeds); pollen when applied to *su* produced 49.8 percent *su*, ear similar to F. H represents *sp*++/+*su lo* ear selfed (0 *su*, 85 seeds), and crossed by *su* (0 *su*, 64 seeds); I=pollen of H on *su* (96.7 percent *su*).

TABLE 3

Percent of sp functioning pollen grains from the cross su×sp +/+ su.

	YEAR SEED COUNTS MADE	% IN C. O. CLASS	YEAR AND PLACE GROWN	TOTAL PLANTS EXAMINED	*sp*/+ NO.	*sp*/+ %	+/+ %	CORRECTED C. O. %
1	1927	9.8	1928 Texas	111	2	1.8	98.2	9.6
2	1926	13.3	1927 Conn.	227	2	6.4	93.6	12.4
3	1929	12.4	1930 Texas	89	0	0	100	12.4
4	1932	10.5	1934 Conn.	116	34	29	71	7.5
5	1932	4.8	1934 Conn.	526	111	21	79	3.8
6	1933	17.5	1935 Conn.	122	67	55	45	7.9
7	1933	8.6	1935 Conn.	1103	135	12	88	7.6
	Total and Average			2294	351	15.3	84.7	
		6.1*	(value for progenies 5 and 7)				84.9	5.2

* Average of all ears counted in Connecticut and Texas.

whose crossover values were obviously high. Two of the populations examined for segregating pollen represented random samples (progenies 5 and 7 in table 3). These two produced a total of 1629 plants of which 246 or 15.1 percent represented functional *sp* pollen instead of recombinations. This left a value of 84.9 percent for recombinations. When this is multiplied by the original crossover value of 6.1, it gives a corrected crossover value of 5.2 percent. This probably represents more nearly the correct value for the recombinations of *su* and *sp* in the repulsion phase.

Our correction factor was based on the number of *sp* pollen grains that not only effected fertilization but *produced mature plants*. Since our experiments have shown a differential viability in favor of *Sp Sp* plants, it is obvious that the number of *sp*/+ plants that survive to the flowering stage is appreciably lower than the seeds of the constitution *sp*+/+*su*. The number of plants lost is not usually known. Any appreciable increase in the number of *sp* functional pollen would further decrease the recombination percentage. In the 1932 ears, the original recombination percent was 4.8. When the crossover seeds were planted in 1935 and the resultant plants were examined for segregating pollen, 21 percent were segregating leaving 79 percent as recombinations. This gives a corrected crossover value of 3.8. This represented several progenies and probably approaches the true crossover value for those progenies.

We are forced to conclude that the original figure of 6.1 percent is slightly too high. Whether there is considerable fluctuation in the recombination percent as well as in the amount of functional *sp* pollen is not possible to determine since we have no accurate way of measuring the *sp*/*Sp* seeds that fail to produce plants. In some cases, at least, we know the value to be as low as four percent and it could be even lower. Since six percent

is the maximum, an average value of five may be taken as the crossover percent. A difference of one percent in the crossing over of these two genes will have little affect on the location of *sp* on the chromosome.

In the coupling phase, the percent of recombinations (plus *sp* pollen survivals) is 9.7 in a total of forty-one thousand. The apparently higher recombination value than for the repulsion phase is probably accounted for largely by *sp* pollen survivals rather than an increased crossover value. This will be discussed more fully under effects upon the male gametophyte.

Linkage relations of lo with su

Like *sp*, the gene *lo* is closely linked with the *su* locus so that its inheritance is studied through its effect on the *Su*:*su* ratios. In table 2 are found the apparent crossover ratios for *lo* and *su* in both the coupling and repulsion phases. Since *lo* ovules are nearly all lethal, the crossover ratio between *lo* and *su* is obtained by backcrossing *lo su*/++ or *lo*+/+ *su* ears by *su* pollen and noting the percentage of "crossover" seeds. The *su* seeds from the cross *lo su*/++ will all be crossovers provided there is no functioning of the *lo su* ovules. This point can be tested in a way similar to that used in testing *sp* survival in the pollen. The "crossover" seeds were grown and the resultant plants examined to determine how many produced *lo*/+ ears and were therefore *lo* ovule survivals instead of recombinations. Table 4 summarizes all "crossover" plants examined.

TABLE 4

Percentage of lo/+ plants produced by "crossover" seeds in both coupling and repulsion phases of lo and su.

GENOTYPE	YEAR	TOTAL PLANTS	*lo*/+ EARS NO.	*lo*/+ EARS %	% ++ EARS	ORIGINAL C. O.	CORRECTED C. O.
lo su/++	1932	88	15	17	83	2.3	1.9
lo su/++	1935	37	22	60	40	1.7	0.7
Total and av.		125	37	30	70	2.3	1.6
lo +/+ *su*	1935	318	220	69	31	5.1	1.6
lo su/+ *su*×++	1933	465	9	1.9*			

* =percentage survival of *lo* ovules compared to *Lo* when each given equal opportunity—no selection of seed (all *su*×*Su*).

In both the coupling and repulsion phases some of the apparent crossover seeds were *lo* ovule survivals. The seeds planted represented random samples of the crossover class. The apparent crossover ratio in the repulsion phase was greater than for coupling of *lo* and *su*. However there were more *lo* ovule survivals in the crossover seeds from the repulsion phases. When both crossover ratios from the progenies whose crossover seeds were examined are corrected, they are exactly the same, 1.6 percent in each case. The "crossover value" obtained in the coupling phase for all years studied

was 2.4 as given in table 2. When this is corrected by multiplying by 70 percent (see table 4) it becomes 1.7 percent, the actual crossover ratio for *lo* and *su* for all years grown. Likewise the total figure for the repulsion phase *lo*+/+*su*, 4.8, when corrected becomes 1.5 percent, the actual recombination percentage for *lo* and *su*.

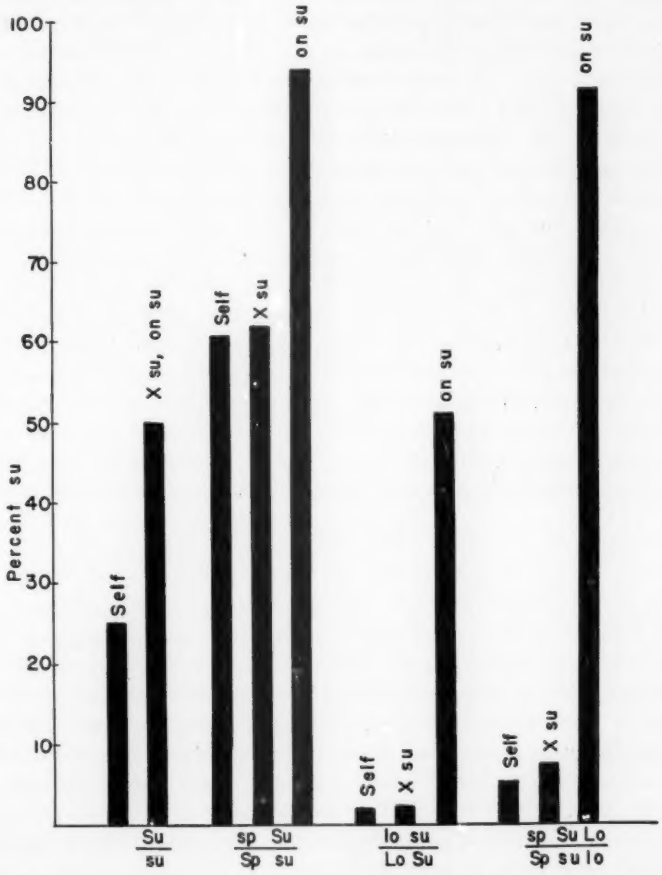

FIGURE 3.—Percent of *su* seeds obtained from self-pollinations and backcrosses to *su* for plants of the compositions *su*/+, *sp*+/+*su*, *lo su*/++ and *sp*++/+*su lo*.

Gametic and zygotic ratios of sp/+ and lo/+ plants

Table 2 gives the gametic ratios (backcrosses to *su*) and zygotic ratios (self-pollinations) produced by plants heterozygous for *sp*, *lo*, and combinations of *sp* and *lo*. This is also shown graphically in figure 3. From the

gametic ratios it is possible to calculate the expected zygotic ratio for comparison with the zygotic ratio found. The expected ratio is given in the eighth column of table 2. By comparing the actual and expected zygotic ratios it is possible to determine whether there is any appreciable differential zygotic mortality between heterozygotes and homozygotes. If there is a regular and appreciable survival of *sp* in the pollen or *lo* in the ovules, there should be some homozygotes produced. If these are eliminated the actual percentage of *su* seeds should be higher than the calculated percentage in the repulsion phase, and lower in the coupling phase. In all cases there is a fairly close agreement between the expected and observed ratios, the differences being noted in the last column of table 2. Some of the differences are negative and some positive. This would indicate no appreciable differential zygotic mortality. To test this point more thoroughly in the case of *sp*+/+*su*, plants from the same progenies were studied in Texas in 1930. Nine progenies were involved which included 255 ears with a total of 57,159 seeds. The total difference for the nine progenies between calculated and observed zygotic ratios was 2.3. In four cases the actual percentage was higher than the calculated and lower in five. All of these data show that differential zygotic mortality is not very influential in disturbing the ratios resulting from selfing heterozygous *sp* or *lo* plants. If there is any differential zygotic mortality it is overshadowed by errors in sampling, differences in crossing over at microsporogenesis and megasporogenesis, and differences in survival of *sp* male gametes.

EFFECTS OF *sp*

Effect on male gametophyte

The effect of *sp* on the male gametophyte was partially discussed in a previous section. The obvious effect is to cause the pollen grains to be reduced in size. In some samples it is possible to count the two classes of pollen grains and in such cases ratios of approximately 1:1 are found (626 normal:643 small in one sample counted). In others, however, there is an overlapping of the two classes. When the distribution of the pollen grains with respect to maximum diameter is plotted as a frequency polygon, the curve for pollen grains from *Sp Sp* plants has only one mode at 96 microns while the curve for *Sp sp* plants is distinctly bimodal with one mode at 84 microns, the other at 99 microns (figure 4).

Of some interest in this connection is the fact that the normal pollen grains from *Sp sp* plants are larger than the grains from normal plants. This is suggested by the frequency distribution in figure 4 and is verified by an actual comparison of all grains with a maximum diameter of 90 microns or more. In *Sp sp* plants these grains average 98.4 microns; in *Sp Sp* plants, 95.3 microns. The difference, 3.1 microns, is probably significant (P

<.03). Evidently the normal pollen grains in heterozygous plants are capable of taking advantage of the reduction in competition resulting from the fact that half of the pollen grains are reduced in size.

Microspores of plants segregating *sp* were examined to see how early the *sp* microspores could be detected. No difference in microspore size or appearance was found at the first microspore division. Just before the second division however, the *sp* grains are slightly smaller and the nucleolus of the tube nucleus is more vacuolated than that of *Sp* pollen grains (figure 5, C and D).

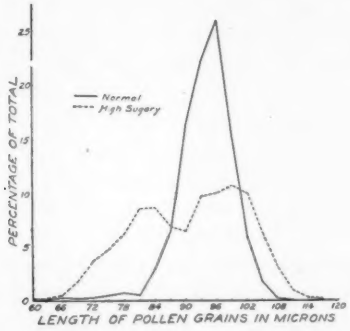

FIGURE 4.—Frequency distribution, with respect to length, of 1350 pollen grains from eleven *sp*+/+*su* (high sugary) plants and 1100 grains from *su*/+ plants.

Although the *sp* grains are smaller than normal they are usually well filled with starch. Preliminary results showed that the *sp* grains rarely effect fertilization in competition with normal grains although they are capable of fertilization when the competition with normal grains is removed by screening (MANGELSDORF 1931, 1932). More recently it has been shown (SINGLETON 1940) that the pistillate parent to which the pollen is applied has considerable influence on the functioning of *sp* pollen. In 1938 the recombination percent of *su* and *sp* was high when pollen of *sp su*/++ was applied to *su*. Crosses on Purdue 39 gave 39.1 percent of *su* seeds, those on Connecticut 81 gave 16.4 percent. Both of these figures suggest that there was considerable functioning of *sp* pollen, more in the case of P39 than for C81. Examination of plants produced by these seeds was made in 1939. The results are given in table 5.

This table shows that the *su* seeds from the P39 cross produced 86.8 percent of *sp*/+ plants while the C81 crosses gave only 55 percent. The corrected crossover ratio for the P39 crosses was 5.2 whereas for the C81 crosses it was 7.4 percent. This difference cannot be a difference in crossing over since pollinations on the two inbreds were made on the same day

TABLE 5

The effect of the pistillate parent upon survival of sp in pollen. Crosses of P39 and C81 by sp su/+ + plants.

PARENTS		NO. SEEDS			PROGENY PLANTS		%	%	CORRECTED
♀	♂	TOTAL	*su*	%*su*	TOTAL	*Sp sp*	*sp*/+	++	C. O.
P39	1174–4	1612	607	37.7	123	111	90.2	9.8	3.7
C81	1174–4	1135	115	10.1	47	32	68.1	31.9	3.2
P39	1175–10	1382	728	52.7	183	165	90.2	9.8	5.2
C81	1175–10	2327	434	18.7	127	58	45.7	54.3	10.2
P39	1176–5	600	156	26.0	114	93	81.6	18.4	4.8
C81	1176–5	1997	350	17.5	91	62	68.1	31.9	5.6
P39	1176–8	864	253	29.3	95	78	82.1	17.9	5.2
C81	1176–8	1365	223	16.3	86	41	47.7	52.3	8.5
Total on P39		4458	1744	39.1	515	447	86.8	13.2	5.2
Total on C81		6824	1122	16.4	351	193	55.0	45.0	7.4

using pollen from the same plants. These results suggest that there might have been a more selective elimination of heterozygous *sp*/+ plants in the case of the C81 crosses. Data are available on this point. These are as follows:

	Seeds planted	Plants obtained and examined	Loss	% loss
P39 crosses	580	515	65	11.2
C81 crosses	512	351	161	31.4
				Difference = 20.2

There was a greater loss from the seed to the mature plant stage for the C81 crosses. If the 20 percent difference in loss is due to elimination of *sp*/+ plants then this amount should be added to the *sp*/+ plants already found for the C81 crosses. Adding this gives 295 *sp*/+ in a total of 453 or 65.1 percent. This leaves 34.9 percent ++ which represent crossovers only. Multiplying this by 16.4, the original "crossover+survival class" gives a corrected crossover value for the C81 crosses of 5.7 which is in agreement with that of the P39 crosses, 5.2 percent. Of course we cannot be sure the additional 20 percent loss for the C81 crosses is all due to elimination of *sp*/+ plants. The fact that *sp*/+ plants tend to be weaker than normal sibs supports the supposition that this is so. At any rate, something has caused the elimination of 31.4 percent of the zygotes between the seed and mature plant stage in the C81 crosses, whereas in the P39 crosses the loss was only 11.2 percent. It seems that the C81 inbred which eliminates more

of the *sp* male gametes than P39 also has a more deleterious effect on zygotes which are heterozygous for *sp*.

In addition to the variable functioning of *sp* pollen grains on different inbreds, and the functioning of *sp* male gametes when screened to remove competition of normal grains, we would expect an increased functioning of *sp* grains if competition were reduced by limiting the pollen so that part of the silks received only one functional grain. We have made no experiments on this point, but data secured from other experiments show that this may occur. Of the 20 ears obtained in 1926 from the backcross *su*×*sp* +/+*su* (89.9 percent sugary) approximately half of the ears were partly filled, some of them undoubtedly so because of insufficient pollen. These ears were arbitrarily divided into two groups, one with less than 200 seeds, the other with more than 200. There were 11 ears in the first group with an average of 127 seeds per ear; nine in the second group with an average of 424 seeds. The percentage of *Su* seeds (crossovers plus *sp* survivals) in the first group was 15.2; in the second, 8.2. The difference is highly significant ($P<.01$). Since the two groups represent random samples of the population in other respects, the differences cannot be attributed to variations in crossing over, and are undoubtedly due to differences in competition between *Sp* and *sp* grains resulting from variations in the amount of pollen available on the styles.

Quite different results were secured in 1930 when a homogeneous population of 55 ears with 94.1 percent of *su* seeds was divided the same way as those in 1926 into two arbitrary classes with more and less than 200 seeds each. In the first group there were 29 ears, in the latter 26. Each group of ears gave an average crossover percent of 5.9. The results of the two tests indicate that competition for limited pollen is undoubtedly a factor in some cases, and not in others.

A third source of variation should be sought in modifying factors which affect the expression of the *sp* gene. Some indication that this occurs is found in the fact that in some samples of pollen from *sp*/+ plants, the small grains can be definitely distinguished from normal grains; in other samples there is an overlapping of the two classes. Yet no correlation between this variation and that in the male gametic ratios has been found, nor is there any correlation between male gametic ratios in two successive generations. The differences are probably due primarily to variations in functioning of *sp* grains rather than to differences in crossing over.

That the variations in "crossing over" are really differences in *sp* pollen survival rather than differences in crossing over is shown by correlating the recombination percent in parent and offspring. If due to a difference in recombination we would expect such differences to be transmitted. The correlation of the crossover percents in parent and offspring is $-.28 \pm .091$

showing no positive correlation and perhaps a slight negative one. The negative correlation is just barely significant.

Effect of sp upon the female gametophyte

The factor *sp* is transmitted largely through the ovules, but there is some lethality of *sp* megaspores. Otherwise, ratios higher than 50 percent *su* could not be obtained for self-pollinations or backcrosses. The figures for these pollinations are 61 and 62 percents based on 143 thousand and 36 thousand kernels respectively. The elimination of *sp* megaspores is also indicated by missing places on heterozygous ears and by the reduced number of kernels compared with *Sp Sp* ears. A comparison of the number of seeds on 61 ++ ears and 237 *sp*/+ ears showed that the *sp*/+ ears had on the average 67.8 percent of the number of kernels on the former ears.

Since there is a semi-lethal action of the *sp* gene upon the megaspores the question arises as to what percent of the *sp* ovules survive and are functional. We can measure directly the percentage of *sp* ovules which accomplish fertilization and produce mature plants. In previously reported three-point tests with *sp su* and *Tu* (EMERSON, BEADLE and FRASER, 1935) 801 plants were grown of which 497 were normal and 304 were heterozygous for *sp*. If *Sp* and *sp* ovules had originally occurred in equal numbers the 304 *Sp sp* plants represent survival for approximately 497 ovules. This is a survival of 61.2 percent.

In the three-point test with *la*, *sp* and *su* reported later in this paper 1898 plants were grown of which 496 were heterozygous for *sp*. Calculated in the same way this represents a survival of 35.4 percent. The weighted average for the two populations is 42.1 percent, which is somewhat less than the 56 percent survival of *sp*[2] reported by RHOADES and RHOADES 1939.

Data already presented indicate that there is sometimes an elimination of heterozygotes between the mature seed stage and the mature plant stage. We can calculate the survival of *sp* through the megaspores to the mature seed stage by the following formula where s is percentage of survival, p = percentage of non-crossovers and m = percentage of functional *su* gametes among the ovules of *sp*+/+*su* plants or the percentage of functional *Su* ovules in *sp su*/++ plants.

$$s = \frac{100(p-m)}{(p+m)-100}$$

If we assume that crossing over between *su* and *sp* is relatively constant at five percent[2] it is found that survival of *sp* through the megaspores to the mature seed stage varies from 0 to 100 percent in different progenies.

[2] Slight differences in crossing over have no appreciable effect upon calculated survival. The results are quite similar if the percentage of crossing over is computed at three percent, the minimum figure, or six percent the maximum.

The average survival in *sp*+/+*su* plants (61.9 percent *su*) is 58.2 percent; in *sp su*/++ plants (37.6 percent *su*) 56.8 percent.

The populations used in the two three-point linkage tests mentioned above permit a direct comparison of survival to the mature seed stage and survival to the mature plant stage. In the three-point test with *Tu* crossing over between *sp* and *su* was 5.8 percent and the percentage of sugary seeds in the backcrossed ears was 61.6 percent. The calculated survival of *sp* to the mature seed stage is 58.4 percent. This is slightly lower than the actual survival to the mature plant stage, 61.2 percent, and indicates that in this particular population there has been no elimination of heterozygotes after the mature seed stage. In the three-point test with *la* the crossing over between *sp* and *su* was 5.0 percent and the percentage of sugary seeds on the backcrossed ears was 36.7 percent. The calculated survival to the mature seed stage is 54.4 percent, which is considerably higher than the actual survival to the mature plant stage, 35.4 percent, and suggests an elimination of heterozygotes. Thus the data not only show extreme variations of survival of *sp* through the megaspores, but support the suggestion already made, that elimination of heterozygotes varies with the stocks used as parents.

The percent of *su* seeds when plants of the constitution *sp*/+ are selfed shows a decided correlation between one generation and the next. The correlation coefficient was $+.40 \pm .04$. This is highly significant. In Texas a similar comparison of six parents and progenies gave a correlation coefficient of $+.67 \pm .23$. Both of these comparisons show that there is a decided tendency for ears that produced a high percentage of *su* seeds one year to give a high percentage the next. Since any percent of *su* seeds in excess of 50 is caused by ovule elimination of *sp* ovules it is evident the degree of ovule elimination is transmitted.

Substitution of megaspores

In maize as well as in many other plants, the embryo sac develops from only one of the four megaspores resulting from the two divisions of the megaspore mother cell. The other three are lost. In several instances where peculiar genetic results have been encountered, it has been suggested that substitution of megaspores may have occurred; that when the megaspore which usually persists, receives a combination of genes with a lethal effect, it may be replaced by a viable sister megaspore. This has been shown by RENNER (1921) to occur in Oenothera and is known as the "Renner effect." Maize is ideal material for investigating this phenomenon because the orderly arrangement of the seeds renders any lethal effect immediately visible.

That the substitution of megaspores in *sp*/+ stocks, if it occurs at all, is not complete, is at once apparent from the fact that ears from hetero-

zygous plants are seldom well-filled and usually exhibit many missing kernels. However, if there is any substitution whatever the number of gaps should be smaller than the number expected to occur on the basis of the calculated survival of *sp* through the megaspores.

In 1926 we compared 29 ears from normal *Sp Sp* plants with 77 ears from *Sp sp* plants. The former had an average of 263 seeds per ear, the latter 179 seeds. Thus, the high-sugary ears had only 68.1 percent as many seeds as normal ears from the same progenies. The average survival of *sp* in 1926 was calculated as 47.4 percent. This means that high-sugary ears in 1926 would be expected to have, on the average, 73.7 percent, $50+(47.4/2)$, as many seeds as normal ears. The difference between this figure and the one actually obtained, 68.1 percent, is easily accounted for as the result of sampling errors. In any case, the difference is in the wrong direction to indicate substitution of megaspores.

In 1930 we compared 160 high-sugary ears with 32 normal ears. The former had an average of 238 seeds per ear, the latter 377. The lower figure is 63.1 percent of the higher. Survival of *sp* in the megaspores in 1930 was computed at 31.0 percent. On this basis ears from *Sp sp* plants would be expected to bear 65.5 percent as many seeds as ears from normal plants. Again the difference is in the wrong direction to suggest substitution of megaspores. The averages for the two years are 69.6 percent calculated and 65.6 percent actually found. That the number of seeds found on ears from *Sp sp* plants is lower in both cases than the number calculated on the basis of survival of *sp* is probably due to the fact that plants heterozygous for *sp* are slightly weaker than normal plants and hence probably bear slightly smaller ears. That the observed and calculated figures agree as closely as they do indicates not only that no substitution of megaspores has occurred, but also that the data on survival of *sp* in the megaspores are reasonably accurate.

Effects of sp upon the sporophyte

Effect upon the heterozygote

That *sp* has a deleterious effect upon the heterozygote has already been suggested by the fact that its survival through the megaspores to the mature seed stage is sometimes greater than survival to the mature plant stage of the succeeding generation. Additional observations support this suggestion.

Plants heterozygous for *sp* are slightly less vigorous and later in blooming than their normal sibs. It is not always possible to demonstrate this fact statistically because of other variables, but it has been observed repeatedly especially in certain progenies. In one season at least (1926), the difference in height of stalk, 2.9 inches, was statistically significant ($P<.03$).

Effect upon the homozygote *sp sp*

Since *sp* survives in part of the megaspores, and *sp* pollen can function, it should be possible to obtain seeds homozygous for *sp*, and if these are viable to determine the effect of *sp/sp* upon the plant. Pollen screening experiments in both Texas and Connecticut have altered the *Su:su* ratio, and have resulted in a considerable functioning of *sp* pollen. So far no homozygous *sp/sp* plant has been found either in ordinary populations or in cases where screened pollen was applied to heterozygous *sp* plants. In one experiment where it was determined that at least 28.7 percent of the functional ovules were *sp* and at least 73.5 percent of the functional pollen grains were *sp* no homozygous plants were obtained in a population of 78 plants derived from 176 seeds. Seedling mortality was high even under ideal conditions which suggests that the homozygous combinations which must have occurred from this pollination were eliminated as seed or seedling lethals.

THE EFFECTS OF *lo*

Effect upon the female gametophyte

The chief effect of *lo* is upon the female gametophyte. The majority of ovules receiving *lo* are lethal, and ears from heterozygous plants are always poorly filled, resembling ears from "semi-sterile" plants in which reciprocal translocations are involved. There is however some survival through the megaspores as was discussed in a previous section. One such test where *lo su/+su* ears were pollinated by *Su* gave 465 ++ ears and nine *lo/+* ears or 1.9 percent of *lo/+* ears. Since this represents an unselected sample it seems safe to say that about two percent of *lo* ovules can take place in fertilizaton.

Effect upon the male gametophyte

There is no visible effect of *lo* upon the male gametophyte, and pollen from *lo/+* plants is quite normal in appearance. Furthermore, there is apparently no elimination of *lo* through the male gametophyte, in fact the contrary seems to be true. In the backcross *su*×*lo+/+su*, the percentage of + seeds was 51.5, while in the backcross *su*×*lo su/++*, the percentage of *su* seeds was 51.3. The total number of seeds in these two populations is 51,606, of which 26,541 or 51.4 percent, represents the class in which *lo* predominates. The deviation from the 50.0 percent expected in this class is 1.4±.148. The deviation is 9.5 times the probable error, and the odds against its chance occurrence are more than a billion to one.

This situation in which a gene is almost completely lethal to the ovules but advantageous to the male gametophytes receiving it, is unique. Perhaps a hormone-like action which stimulates the male gametophyte and retards the female gametophyte is involved.

Effect upon the sporophyte

We have never noticed any effect of *lo* upon the sporophyte. Plants of the constitution *lo*/+ seem to be as vigorous as those of the constitution +/+ and there is no noticeable difference in time of flowering.

No plants homozygous for *lo* have been obtained. It should be possible to obtain such individuals since about two percent of the functional ovules are *lo* and since the *lo* pollen functions normally. An attempt will be made to obtain homozygous *lo*/*lo* plants.

LINKAGE RELATIONS OF *sp* AND *lo*

Linkage relations of sp

In a previous section we have found the recombination value for *sp* and *su* to be approximately five percent.

TABLE 6

Three-point linkage tests with sp and lo.*

F_1 GENOTYPE	PARENTAL COMBINATIONS		RECOMBINATIONS REGION 1		REGION 2		REGIONS 1 AND 2		TOTAL
Ts5 + + / + *sp su*	738	—	— 50		10	—	— 4		802
			6.2		1.2		.5		
Ts5 sp + / + + *su*	—	367	77	—	—	45	24	—	513
			15.0		8.8		4.7		
Total	1105		127		55		28		1315
			9.7		4.2		2.1		
la + +†/ + *sp su*	781	439	781	14	41	16	2	35	1898
lo + + / + *su gl3*	1748		161		1048		52		3009
			5.4		34.8		1.7		

* For additional tests already published see EMERSON, BEADLE and FRASER 1935.

† Backcross for *su* and *sp* F_2 for *la*.

Various three-point tests with *sp su* and *Tu*, the results of which have already been published (EMERSON, BEADLE and FRASER 1935), show *sp* to be to the left of *su*. Several three-point tests with *Ts5 sp* and *su*, show *sp* to lie to the right of *Ts5* and to the left of *su*. The data are shown in table 6.

The three-point test for *la*, *sp* and *su* given in table 6 proved to be an F_2 for *la* and a backcross for *sp* and *su*. Evidently a +*su*/*la su* plant instead of the double recessive was used for a pollinator. The cross was then *la*++/+*sp su*×*la su*/+*su*. The crossover value for the three genes is as follows: *la* 7.5 *sp* 5.0 *su*. The recombination value for *la* and *su* was 13.2 percent. The crossover values for *la* and *sp*, also for *la* and *su* were calcu-

lated by the product method (IMMER 1930) using the formula ad/bc $=p+p^2/2-3p+p^2$ where p equals the crossover value. The crossover value for *sp* and *su*, 5.0 percent, was calculated directly since this was a backcross for both, and survival of *sp* in the pollinations not involved.

The only data which suggest that *sp* might be to the right of *su* are those showing the crossing over between *sp* and *gl*3 to be 26 percent. Published data (EMERSON, BEADLE and FRASER 1935) show crossing over between *su* and *gl*3 to be 34 percent, and the much lower crossing over between *sp* and *gl*3 would suggest that *sp* lies between *su* and *gl*3. Since all three-point tests show that the contrary is true, this is another example of the variations involved in comparing crossover values from different stocks.

One additional linkage test with *sp* should be mentioned because of the peculiar ratios obtained. The gene *de16*, which causes defective seeds, is located on the fourth chromosome about 3.2 units to the right of *su*. Plants of the composition *sp* ++/+ *su de16* should, when selfed, be high-*su* and high-*de16* seed. In most ears, however, the *su* gene does not express itself in seeds homozygous for *de16*, and since *su* and *de16* are closely linked, there is actually a decided deficiency of visible *su* seeds. In a total population of 2609 seeds from 11 ears, 1430 or 54.8 percent of the seeds were *de16*, while 106 or 4.1 percent of the total were visibly *su*. Yet when pollen from one of these plants was applied to normal *su* plants the percentage of *su* seeds produced was 90.6. A similar situation has been described in which the gametic lethal *lo* is involved.

Among those 11 ears was one in which the + and *su* endosperms in the defective seeds were distinguishable. The four classes of seeds occurred in the following numbers: ++, 117; +*su*, 21; *de16* +, 15; and *de16 su*, 67. Since 40.0 percent of all seeds are *su* and 37.3 percent are *de16*, the results suggest that *sp* has had a greater effect upon *su* than upon *de16* and hence must be closer to *su* than to *de16*. Thus these data, though not critical, are in agreement with other three-point tests which place *sp* to the left of *su*.

Linkage relations of lo

It has already been shown that the percentage of recombinations for *lo* and *su* in the ovules is 1.6 percent. Likewise in published data (EMERSON, BEADLE and FRASER 1935) the recombination percent in the pollen for the repulsion backcross was 1.4 percent. This is very close to the value for ovules. Since the publication of that figure a few additional progenies have been summarized which may alter slightly the recombination percent observed from the pollen. All data are summarized in table 7.

These values are variable especially in the coupling phase. Not much reliance is placed on the figures for this phase since the figures for the most part represent compilations of small progenies.

TABLE 7

Recombination values from backcrosses of lo and su in pollen.

	REPULSION PHASE				TOTAL	RECOMBINATIONS	
	++	+ *lo*	*su* +	*su lo*		NO.	%
1	1	85	80	0	166	1	0.63
2*	1	444	78	2	525	3	0.57
3*	9	190	185	1	385	10	2.6
4	0	92	124	18	234	18	7.7
5	11	265	288	7	571	18	3.2
6	5	160			165	5	3.1
7	23	805	776	24	1628	47	2.9
Total	50	2041	1531	52	3674	102	2.8
			COUPLING PHASE				
8	34	2			36	2	5.6
9	197	19	19	179	412	38	9.2
10	52	15	9	50	126	24	19.0
11	44	0	0	45	89	0	0
Total	327	36	28	274	665	64	9.7

* The totals of 2 and 3 give an average of 1.4 percent, the figure published by EMERSON, BEADLE and FRASER 1935.

In the repulsion phase, the average of all years' trials before 1939 is 2.7 percent. This is higher than the values found from the ovules after correcting for *lo* ovule survival. The values obtained for different progenies vary considerably, from .57 percent to 7.7 percent for one progeny of 234 plants where all the recombinations were of the *su lo* class and there were none of the ++ type. Omission of this progeny from the calculations brings the average figure to 2.4 percent. In four of the progenies, totaling 681 plants, in the repulsion phase the crossover value was less then one percent. It is evident there is considerable variation in the crossover values from the pollen. Part of this might be caused by mistakes in classification, as all *lo*/+ plants were classified entirely by the semi-sterile appearance of the ear. Although on the whole this is a reliable criterion, in the case of ears poorly filled for other reasons there may have been a few mistakes. Also, crossovers of the constitution ++ could have arisen from a contamination in the original pollination. In the 1939 data it is doubtful if this could have been a factor since extreme care was taken to guard against contaminations in the original pollinations. The fact that both crossover classes are numerically so nearly alike leads us to believe that errors in classification were negligible in these progenies.

The conclusions regarding the percentage of recombination between *lo* and *su* are that in the pollen the percent for the repulsion phase varies from one to three with an average of 2.8. The recombination value in the ovules for both coupling and repulsion phases was 1.6. There may be a dif-

ference in the amount of crossing over in microsporocytes and megasporocytes. Since the variation of the crossover values is from 1.6 to 2.8, no serious error will be encountered if any value between those figures is taken as the recombination value for *lo* and *su*. If we take the mid-way point for these two figures we get a value of 2.2 percent (which is the average value published in 1935 by EMERSON, BEADLE and FRASER). The crossover value of 2.2 will be used for *lo* and *su* in calculating linkage with other factors on chromosome 4.

Recombination value of *lo* and *Ts5* is eight percent, and there are two percent of recombinations between *lo* and *de16* (EMERSON, BEADLE and FRASER 1935). Other linkages of *lo* on chromosome 4 are shown in table 8.

TABLE 8

Crossover values of sp and lo with gl3.

GENES X AND Y	LINKAGE PHASE	XY	Xy	TOTAL	RECOMBINATIONS NO.	RECOMBINATIONS %
Lo Gl3	RB	1722	2924	4646	1722	37
Sp Gl3	RB	1210	3530	4740	1210	26

This shows 37 percent of recombinations between *lo* and *gl3*. This would tend to place *lo* to the left of *su*, since *gl3* and *su* show 34 percent of recombinations. However, where two factors are as far apart as 34 units they are not very useful in locating a third gene which is close to one of them. The data with *de16* are more conclusive. This lethal shows 3.2 percent of recombinations with *su* and the value for *lo* and *su* is 2.2 percent. Hence if *lo* were on the left of *su* we should expect about five percent of recombinations between *lo* and *de16*. Actually there was two percent of crossing over between *de16* and *lo* which would be expected if *lo* were to the right of *su*. These data agree with the three-point test between *sp*, *su* and *lo* placing *lo* to the right of *su*. One three-point test for *lo*, *su* and *gl3* was summarized in table 6. The crossing over between *lo* and *su* is 7.1 percent; between *su* and *gl3*, 36.5 percent; between *lo* and *gl3*, 40.2 percent. These data were obtained from crosses in which *lo* survival in the ovules could have been a disturbing element. It is impossible now to correct for that factor although the 7.1 percent of crossovers between *su* and *lo* indicate a survival of *lo* ovules. Even if corrected it would not alter the relative positions of the three factors calculated from these data. This three-point test tends to place *lo* to the left of *su*, in direct conflict to the three-point data of *sp*, *su* and *lo* and emphasizes the variations found in this study.

Combinations of sp, lo and su

The combination of sp $++/+$ *su lo* gives the unusual result described in the introduction and shown graphically in figure 3. Actual ratios are

found in table 2. At the time *lo* was discovered it was not known on which side of *su* it was located. If on the same side it would not be improbable that the two were alleles of the same gene. However a simple test rules this out and also gives valuable data on the location of *lo* in relation to *su*. The pollen from a plant, *sp* ++/+ *su lo*, was applied to *su* silks. If *lo* and *sp* were alleles of the same gene then the progenies should either segregate *lo* or *sp*, and no normal ratios should be found. The cross of *su* by *sp* ++/+ *su lo* gave 33 selfed ears. Thirty-one segretated in a normal 3:1 ratio for *su* and two segregated for *sp*. No *lo*/+ ears were found. The occurrence of the 3:1 ratios proves that *sp* and *lo* are not allelic. These ratios also tend to show that *lo* and *sp* are on opposite sides of *su*. If on the same side, the composition would be *sp* ++/+ *lo su* with the crossover values *sp* 2.8 *su* 2.2 *lo* since *sp* is five units from *su*, and *lo* and *su* are 2.2 units apart. Hence we should expect the crossovers at region 2 to be almost equal to those at region 1, 2.8:2.2 percents respectively. The functional male gametes should be +++ (56 percent) and + *lo* + (44 percent). Only *Su* seeds were planted, so *su* gametes need not be considered. A similar comparison of *su* seeds from the cross *su*×*sp su*+/++ *lo* gave similar results. Both sets of data are summarized below.

	Crossovers	
	Region 1	Region 2
su×*sp*++/+*lo su* (*Su* seed)	31	0
su×*sp*+*su*/+*lo*+ (*su* seed)	200	12
Total	231	12
Expected (56%:44%)	136	107

This deviation is highly significant ($P < .01$). The crossovers at region 2 were far too few. If *sp* and *lo* are on opposite sides of *su* then we would expect very few of the second class, double crossovers only. Although not conclusive, these data strongly suggest that *sp* and *lo* are on opposite sides of *su*.

In 1939 a three-point test was conducted for *sp*, *su*, and *lo*. The 212 plants just described were taken from this three-point test. Complete results are presented in table 9. In planting this test it was not possible to grow all the seed available and no effort was made to grow the same relative amounts of *Su* and *su* seed that occurred in the original pollinations. We were more interested in the plants produced by the *su* seeds so relatively larger amounts of the *su* seed were planted. In calculation it was necessary to correct for the *su* seed grown, reducing the *su* seed to the same proportion found in the original crosses. This correction factor is given in table 9.

Also there was some elimination of *sp* male gametes, more in the C81 crosses than in the P39 hybrids. In order not to discriminate against the *sp*

classes it was necessary to increase the individuals in these classes. This was done by dividing the *sp* class found by the percentage of *sp* individuals found, 86.8 percent in the P39 crosses, and 56.4 percent in the C81 crosses.

The use of this correction factor may be questioned but we believe by using it the recombination values are much more accurate than would have been obtained had no correction factor been used. It would be desirable, of course, to have additional data from experiments where no correction factor was necessary.

TABLE 9

Three-point test for sp, su and lo. F_1 genotype=sp su +/++ lo.

	P		R 1		R 2		R 1 and 2		TOTAL
	sp *su* +	+ + *lo*	*sp* + *lo*	+ *su* +	*sp* *su* *lo*	+ + +	*sp* + +	+ *su* *lo*	
P39×1174–4, 1176–5, 1176–10	259	272	4	31	7	4	4	4	
P39 corrected for *su* seed planted.*	138	272	4	16.5	3.7	4	4	2.1	
P39×1176–8	65	99	1	11	1	0	0	3	
P39 corrected for *su* seed†	42	99	1	7.1	.6	0	0	1.9	
Total P39 crosses corrected for *su*	180	371	5	23.6	4.3	4	4	4	
Total corrected for *sp*=*sp* class/86.8	207	371	5.8	23.6	5.0	4	4.6	4	
	578		29.4		9.0		8.6		625.0
			4.7%		1.4%		1.4%		
C81 crosses	210	355	7	15.8	4	9	9	5	
C81 corrected for *su*¶	36.1	355	7	27.2	.7	9	9	.9	
C81 corrected for *sp*=*sp* class/56.4	64.0	355	12.4	27.2	1.2	9	16.0	.9	
	419		39.6		10.2		16.9		485.7
			8.2%		2.1%		3.5%		
Total	997		69.0		19.2		25.5		1110.7
			(6.2%)		(1.7%)		(2.3%)		

* Seeds in ratio of 2714 *Su*:1744 *su* planted in ratio of 120:145
Correct number of *su* should have been 120:X::2714:1744=77.1 seeds.
77.1/145=53.2 percent *su* class×53.2 percent=corrected *su* class.
† *Su*:*su* planted=145:145
¶ Seeds in ratio of 7089:1478 planted in ratio of 120:145

A comparison of the recombination values in table 9 shows 6.2 percent for *sp* and *su;* 1.7 percent for *su* and *lo* and 2.3 percent of double crossovers, more than the crossover percent for region 2. This suggests that *sp* and *lo* are on the same side of *su*. Either way the experiment is calculated, the percent of double crossovers is much too high and suggests either faulty classification or that some other factors such as pollen contamination or hetero-fertilization may have tended to increase the individuals in the double crossover class. We have encountered several cases of apparent hetero-fertilization during the course of this investigation. If any of the seeds in the double crossover class were cases of hetero-fertilization they would belong in the parental rather than the double crossover class.

From all the data we would be forced to conclude that it is not possible to tell whether *sp* and *lo* are on the same or opposite sides of *su*. However the most critical part of the data, as well as previous data, strongly suggest opposite sides, as was discussed previously.

CYTOLOGY OF *sp*/+ AND *lo*/+ PLANTS

In regard to the unusual behavior of the *sp* and *lo* genes, it is important to know if any cytological disturbance is present. In a preliminary report (SINGLETON 1932) it was stated there were "no conspicuous irregularities of chromosomes at the reduction division." This referred to examination of the chromosomes in mid-prophase of meiosis. Since that first examination of the pachytene chromosomes, STADLER (1933) has demonstrated a case of small pollen in maize associated with a deficiency in chromosome 10. STADLER's small pollen behaved genetically similar to *sp*. Since these cases are so much alike, reexamination of chromosome 4 in a number of plants known to be segregating for *sp* was undertaken. A greenhouse progeny in Connecticut was examined cytologically in 1939. Four plants, heterozygous for *sp*, later verified by pollen examinations, were examined at mid-prophase of the first meiotic division. No deficiency was observed in any plant, and pairing of all chromosomes was normal. The factors *sp*, *lo* and *su* are on chromosome 4 which was studied particularly for any evidence of a chromosomal alteration. Pachytene chromosomes have also been repeatedly examined in Texas in plants heterozygous for *sp*, and likewise no deficiency was observed. Plants heterozygous for *sp2* on chromosome 10 also showed no visible deficiency. (RHOADES and RHOADES 1939.)

Two plants, heterozygous for *lo*, were examined in Connecticut in 1939. No deficiencies were observed in any of the chromosomes 4 examined, and all other chromosomes were apparently also normal (figure 5).

DISCUSSION

There are now on record many occurrences of maize plants segregating small pollen, some spontaneous, others induced by X-rays or by ultraviolet radiation. In some instances these may be associated with a visible chromosomal deficiency (STADLER 1933) but in most of the cases so far observed no chromosomal irregularities can be detected. In most segregations for small pollen, the smaller grains are comparatively unable to effect fertilization in competition with the normal larger grains. This condition has been observed so often that it is now regarded as of general occurrence that the small pollen grains are not capable of competing with the larger grains.

In our study of *sp* several observations have shown a considerable proportion of *sp* grains that accomplished fertilization in competition with

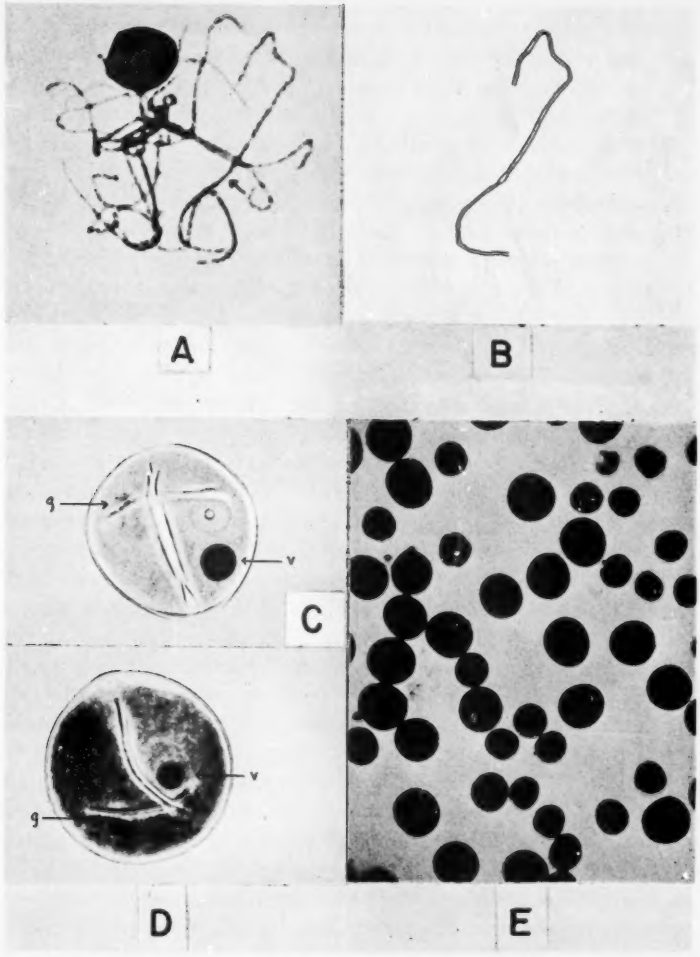

FIGURE 5.—A, pachytene preparation from *sp*+/+*su* plant showing chromosome 4. Arrow points to centromere. No evidence of lack of pairing or heterozygous deficiency. B, drawing of chromosome 4 in A. C, small pollen-1 microspore before division of generative cell. v = nucleolus of vegetative nucleus, g = generative nucleus. Note vacuolated condition of nuceolus of vegetative nucleus. D represents a normal microspore from same plant as C which was segregating *sp*. Note larger size of microspore, denser cytoplasm and smaller but more compact nucleolus of the vegetative nucleus. D is more completely filled with starch at this stage. E shows pollen from *sp*+/+*su* plant stained with iodine. Smaller grains in most cases well filled with starch.

normal; in pollination from one plant in 1938 there was no competitive effect whatever. Also, the stock to which the pollen is applied can make considerable difference in the amount of small pollen functioning.

So far as we are aware, no gene mutations have been found responsible for any gametic lethals in maize. The *sp* reported by MANGELSDORF for the first time in 1931, was the first case of a partial gametic lethal to be observed that was not associated with a visible chromosome abnormality. It is possible that *sp* may be caused by an undetectably short deficiency, but as stated before there is no visible alteration of chromosome 4 in stocks segregating for *sp*. If the abnormality is undetectable, we have no way of knowing whether it has a cytological basis or whether it is in the nature of a gene mutation.

In some ways it behaves like a gene mutation: it is transmissible through the ovules, in some cases with very little or no ovule elimination; in some instances, when pollen from a heterozygous plant is used, the *sp* grains function almost as well as the normal grains. Usually, however, there is almost complete elimination of *sp* grains, the lethal action being complete from a functional standpoint. In linkage relations with characters on chromosome 4, *sp* also behaves like an ordinary gene.

In one important respect the *sp* factor definitely behaves as a deficiency: plants heterozygous for *sp* are quite often smaller than normal sibs. This was especially true when the stock was first grown in 1926. Also one progeny grown in 1927 showed several abnormally small plants. At various other times we have noted that plants heterozygous for *sp* were later in maturity and not as vigorous as sib plants. In this way the factor *sp* behaves more like a chromosomal deficiency than like an ordinary recessive gene. We know of no convincing evidence indicating that a recessive gene in the heterozygous condition causes the plant to be noticeably smaller than the homozygous dominant. All normal recessive genes tested have given just as vigorous plants in the heterozygous condition as do the normal sibs. MANGELSDORF (1928), KARPER (1930), and ROBERTSON (1932) have shown that recessive seed and seedling lethals in maize, sorghum, and barley, respectively, have no measurable effect upon the heterozygote. The *sp* factor seems to be a border line case, cytologically invisible, which acts both like a gene and a deficiency.

This condition is similar to the situation in Drosophila. SLIZYNSKA (1938) analyzed the salivary gland chromosomes of 14 genetic deficiencies in the white-facet region. These are all lethal when homozygous. The longest of the cytological deficiencies included 45 bands, five included one band and one was undetectable cytologically. This last case is like the *sp* condition. This deficiency in Drosophila was a zygotic lethal whereas *sp* is a functional gametic lethal, as well as a zygotic lethal.

SLIZYNSKI (1938) studied 19 spontaneous lethals and 13 lethals induced by X-rays. Of the spontaneous lethals, nine were associated with a cytological deficiency and four of the 13 induced cases were visible cytologically. In more than half of all the cases studied, however, no deficiency in the salivary chromosomes was visible. The reverse condition is shown by DEMEREC and HOOVER (1936). They found a visible cytological deficiency having no genetic effect. DEMEREC (1940) has also made quite an extensive study of X-ray induced and spontaneous Notches in Drosophila. In 27 X-ray induced Notches, three had the full complement of bands, in seven cases one band was missing, in four cases two to five bands were missing and in 13 cases more than six bands were missing. In 10 spontaneous Notches there was one with no bands missing, and nine with one to more than six bands deficient. Thus, lethals and detectable chromosomal deficiencies, although sometimes associated, are not necessarily so.

The *sp* condition in most cases is a functional lethal although the gametes are produced, and the lethality is not necessarily complete. The *lo* factor, however, is a true gametic lethal, the action taking place early in the development of the female gametophyte. Ovule elimination is almost complete for *lo* ovules. In no cases have we found more than a very small percent of the *lo* ovules functioning. This is the only gene of its kind in maize, with its lethal action confined exclusively to the ovules. All other conditions, so far described, that affect the gametes have a more pronounced effect on the male than on the female gamete. Chromosomal disturbances, deficiencies and duplications, have this affect. The *lo* factor however, exercises its lethal affect solely on the ovules. If there is any affect on the male gametes it is one of stimulation since the ratios when pollen from *lo*/+ plants is applied to normal stocks there is an excess functioning of *lo* gametes. Nothing is known of the mechanism whereby *lo* pollen grains are favored over the normal. There seems to be some selective action, making the *lo* gene act as a male influence.

SUMMARY

1. The gene *sp* (small pollen) causes pollen grains to be reduced in size. In plants heterozygous for *sp* two sizes of pollen grains are produced in approximately equal numbers.

2. Pollen grains carrying the *sp* gene usually do not function in competition with normal grains, hence the gene is usually not transmitted through the pollen. When competition between + and *sp* grains is reduced or eliminated as a result of sifting the pollen or because of a sparsity of pollen on the styles, the *sp* pollen grains are capable of accomplishing fertilization. Pollen grains carrying the *sp* gene also regularly function successfully on the styles of certain maize stocks.

3. The *sp* gene is transmitted through part, but not all, of the female gametes; survival of *sp* through the megaspores varies from 0 to 100 percent. The average survival is 42.1 percent to the mature plant stage of the succeeding generation.

4. Plants heterozygous for *sp* are usually slightly weaker than their normal sibs. Heterozygous seeds are slightly smaller and frequently germless.

5. The gene *sp* is a seed or seedling lethal in the homozygous condition; no mature homozygous plants have ever been obtained.

6. The *sp* gene is linked with the *su* locus with approximately five percent of crossing over. In the repulsion phase this results in high-*su* ratios, selfed ears producing 61 percent of *su* seeds and backcrosses by and on *su* producing 62 and 93.9 percent of *su* seeds, respectively. In the coupling phase the ears are low-*su* and the corresponding percentages are 3.2, 38 and 9.7, respectively.

7. Although *sp* has many of the characteristics of a haplo-viable deficiency, cytological studies have failed to disclose such a chromosomal condition.

8. The gene *lo*, which appeared in a stock segregating for *sp*, is a gametic lethal with unique effects. It eliminates practically all the megaspores which receive it but has no effect, or a slightly stimulating effect, upon the male gametophyte.

9. The *lo* gene is also linked with the *su* locus, crossing over being approximately two percent between *lo* and *su*. In the repulsion phase the percents of *su* seeds resulting from selfing and backcrossing by and on *su* are 47.1, 95.2, and 48.5, respectively. In the coupling phase the corresponding percents are 1.8, 2.4, and 51.3, respectively.

10. The *lo* gene is not allelic to *sp*. Plants heterozygous for *sp*, *lo*, and *su* produce 5.2 percent of sugary seeds when selfed, 8.5 percent when backcrossed by *su*, and 91.6 percent when backcrossed on *su*.

11. Various linkage tests with other genes on chromosome 4 show that the order of the genes is probably *Ts5 la sp su lo de16 Tu gl3*.

We are indebted to MISS FRANCES CLARK and DR. R. G. REEVES for preparation and examination of part of the pachytene material. Also, MISS CLARK found the differences observed in the microspores between *sp* and +.

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THE EFFECT OF AN EARLY LETHAL (t^0) IN THE HOUSE MOUSE

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INTRODUCTION

THREE strains of mice in which mutations have brought about malformations of the tail have proved to be of special interest for developmental studies. These mutations affect very early stages of embryonic life, and have thus made possible the experimental analysis of some early developmental steps in mammals which had not before been open to attack, because of experimental difficulties. The three factors involved are probably alleles (they do not show any crossing over), and their effects on early development are not the same. Thus, they offer good material for a study of the relationship of the developmental effects of three unilocal factors.

One of the three strains of mice concerned is short-tailed, and two are tailless. The development of the short-tailed heterozygote ($T+$) and the homozygote (TT) which dies on the 11th day of intrauterine life, have been described by CHESLEY (1935). He found the abnormalities in both the heterozygote and the homozygote to center around a deficiency of the notochord. This structure is nearly completely absent in the homozygote TT and is missing in that part of the tail of the heterozygote $T+$ which is to be constricted and resorbed in the latter half of embryonic life.

The genetical analysis of tailless line A, discovered by DOBROVOLSKAIA-ZAVADSKAIA and KOBOZIEFF (1927) proved it to be heterozygous for T and t^0 (CHESLEY and DUNN 1936). Matings of Tt^0 by Tt^0 produce only tailless progeny at birth; actually, three types of embryos are formed of which only Tt^0 (tailless) is viable while TT dies on the 11th day of pregnancy, and t^0t^0 dies shortly after implantation. The maintenance of the line as a balanced lethal system is due to the absence of crossing over between the two lethal factors T and t^0. The second tailless line (29) is heterozygous for T and t^1 (DUNN 1937) and behaves similarly to Line A, breeding true for taillessness and yielding three types of embryos, TT, lethal on the 11th day, Tt^1, tailless and viable, t^1t^1, lethal before implantation (GLUECKSOHN-SCHOENHEIMER 1938b).

The study of the development of the tailless embryos in lines A and 29 has shown that failure of the notochord in the tail is probably the chief cause for the resorption of the tail in later stages.

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A study of the effects of t^0t^0 on the embryo seemed to be of special interest, first because it produces its effect in a very early embryonic stage, immediately after implantation of the embryo, and second, because the manifestation of other mutations at this locus (TT, $T+$, Tt^0) appears to involve early changes in some of the primary structures such as the notochord and the neural tube, and it is thus important to determine whether the earlier effect of another combination of factors at this locus (t^0t^0) changes the development of structures antecedent to the notochord and neural tube.

The following investigation was undertaken as a continuation of CHESLEY's studies on the t^0 homozygote. From examinations of pregnant uteri from tailless by tailless matings, on the sixth, seventh and eighth day of pregnancy, CHESLEY assumed that resorption of the abnormal type began on the sixth day and was completed by the eighth day. In the present study, it was possible to find the lethal embryos before their death and thus to study their morphological characters and the exact time of death.

The author wishes to thank DR. L. C. DUNN for the suggestion of the problem and for his aid and advice during the progress of the work.

MATERIAL AND METHODS

All animals used in these experiments were from the first to fifth backcross of DR. ZAVADSKAIA's original stock (A line) to an inbred stock of Bagg albinos. The embryos were timed by the vaginal plug method. Male and females were left together for two to six hours and the females then were examined for vaginal plugs. The age of the embryos was thus known within ± 1 to ± 3 hours. The embryos were obtained from the mother as described by GLUECKSOHN-SCHOENHEIMER 1938a. Some of the embryonic capsules were dissected for diagnosis of possible gross malformations (40 embryos from four litters), but the rest of them (239 from 25 litters) were preserved and studied histologically. The embryos were fixed in Bouin's fluid, imbedded in paraffin and sectioned at 8 μ. The embryonic capsules were sectioned longitudinally. The orientation of the embryo within the capsule is not constant enough to permit of the determination of sagittal and frontal sections at imbedding. It is, however, possible to distinguish between the long and short axis of the embryonic capsule; as the embryo itself is usually orientated in mesometral-antimesometral direction (that is, along the long axis), it is thus possible to distinguish between cross and longitudinal sections. But it has to be left to chance whether sagittal or frontal sections are obtained.

FREQUENCY OF ABNORMAL EMBRYOS

Table 1 presents a summary of all dissections. Matings of $+t^0$ females by $+t^0$ males or of $+t^0$ females by Tt^0 males were used. Twenty-nine pregnant

females were dissected on various days after fertilization and a total of 279 embryos was obtained. Of these, 32 were dissected before the time when a diagnosis of typical t^0t^0 is possible. Of the remaining 247 embryos, 162 were normal and 85 abnormal. The ratio of normal to abnormal shows a significant departure from the expected 3:1 ratio, an excess of abnormals being found (35.1 percent instead of the expected 25 percent). This agrees with the results of CHESLEY and DUNN (1936) who in the genetic analysis of the A line observed an excess of offspring with t^0 wherever t^0 is introduced from the male. Their dissections of tailless females pregnant by tailless males revealed an excess of t^0t^0 offspring. From dissections of matings of $+t^0$ by $+t^0$ they reported 35.6 percent of the lethal type t^0t^0, instead of the expected 25 percent. The same phenomenon was observed in another tailless strain, line 29, carrying T and another allele, t^1. In this line also there was always an excess of offspring carrying t^1 wherever t^1 was introduced from the male, as discussed by DUNN and GLUECKSOHN-SCHOENHEIMER (1939).

TABLE 1

AGE				NO. AND TYPE OF LITTERS	TOTAL	NORMAL	ABNORMAL	%
DAYS	HOURS	DAYS	HOURS					
4	23	– 5	3	3($+t^o \times +t^o$)	32	29	3	9.4
5	5	– 5	22	3($+t^o \times Tt^o$)	28	19	9 (1 atypical)	32.1
5	18	– 5	23	4($+t^o \times +t^o$)	28	20	8	28.6
6	0	– 6	20	8($+t^o \times +t^o$)	81	57	24 (4 atypical)	29.6
6	3			1($+t^o \times Tt^o$)	9	6	3 (1 atypical)	33.3
7	2	– 7	3	3($+t^o \times +t^o$)	31	18	13 (2 atypical)	41.9
7	3	– 7	22	3($+t^o \times Tt^o$)	34	21	13	38.2
8	0	– 8	3	4($+t^o \times +t^o$)	36	21	15	41.7
Grand total				29	279	191	88	31.9
Total classified*				26	247	162	85	35.1

* Total embryos aged 5 days 5 hours to 8 days 3 hours; that is, those in which lethals could be distinguished.

The earliest litters examined in the present experiment were between the ages of four days and twenty-three hours and five days and three hours. During that time the difference between normals and lethals is not yet apparent; the few abnormals found probably resulted from accidental causes. From the age of about $5\frac{1}{4}$ days on, a group of embryos is histologically clearly different from the normals and can be classified easily. In frequency this group corresponds roughly to the proportion of homozygotes expected. The percentage of this type of abnormal embryos found from the beginning to the end of the sixth day is around 30 percent, both in matings of $+t^0$ by $+t^0$ and of $+t^0$ by Tt^0. At the age of six days the percentage of abnormal embryos also is around 30 percent while at the age of seven and

eight days it is found to be higher, namely around 40 percent. At that age the lethal embryos are dead and in the process of being resorbed; their classification is thus not based upon any distinct histological differences. In the group of "dead and resorbed" at the age of seven and eight days some embryos may have been included which genetically were not t^0t^0 but had died from accidental causes. Of the 88 abnormal embryos found in these dissections, eight were classified as atypically abnormal, that is, they did not show the characteristic features of the majority of abnormal embryos. It cannot be ascertained whether or not these embryos fall into the class of lethals. But even when they are subtracted from the total, the percentage of abnormals does not change significantly.

EARLY DEVELOPMENT OF THE NORMAL MOUSE EMBRYO

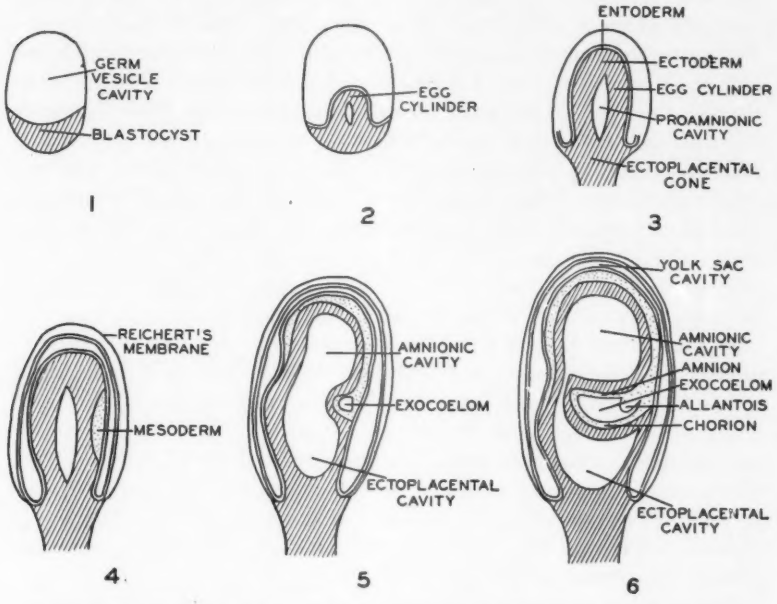
A short account of the early embryogeny of the mouse is given here for comparison with the description of the abnormalities in the homozygote t^0t^0.

The early stages of the embryogeny of the mouse up to the eighth day after fertilization have been described very thoroughly by LEWIS and WRIGHT (1935) (preimplantation stages) and by SOBOTTA (1895, 1903, 1911). Our own observations start at the time of implantation of the embryo, and agree perfectly with those of SOBOTTA.

Fertilization takes place in the Fallopian tube. During the first and later stages of cleavage the egg stays in the oviduct. The two cell stage is attained at about 24 to 36 hours, the four to eight cell stage at 44 to 50 hours, the eight-cell to morula stage at 50 to 69 hours. The egg is very small, and the morula consists of very small cleavage cells. At about the 32 cell stage (that is, approximately the beginning of the fourth day) the eggs enter the uterus and begin to disperse throughout it. In this stage (the second half of the fourth day) the cleavage cavity within the egg forms; it is multiple, eccentric and irregularly shaped. At about the beginning of the fifth day the cleavage cavity of the blastocyst becomes rounded off and regular, and the blastocyst is now spherical.

From now on a considerable increase in cell number takes place, the germ vesicle becomes ellipsoid and the largest part of the wall of the germ vesicle touches the decidua of the uterus. This stage is reached on about the first half of the fifth day (figure 1). Implantation does not take place until the end of the fifth day. Up to that time development is very slow. Only after implantation (that is, after one third of the entire gestation period has passed) does development begin to proceed with great speed. From this stage on, SOBOTTA's observations have been confirmed by our own. Discrepancies in the determination of the age of the embryos are probably due to the fact that we timed matings within a shorter time limit than SOBOTTA did.

At the end of the fifth day the mesometral wall of the germ vesicle begins to thicken and the cells begin to grow both into the germinal cavity in the shape of a cone and into the lumen of the uterus (figure 2). The cone-shaped mass of cells becomes the egg cylinder. The cells of the egg cylinder displace the former germ cavity; this is the beginning of the phenomenon known as entypy or inversion of germ layers, which results in the ectoderm becoming the innermost and the entoderm the outermost layer of the em-



FIGURES 1–6.—Diagrams of different stages in early mouse development, from implantation stage to formation of mesoderm. For detailed description see text.

bryo. At the mesometral pole of the blastocyst the ectoplacental cone is formed (figure 3). In the egg cylinder a cavity develops (figure 3). The entire cylinder and the inner wall of the germ vesicle are now covered by one layer of yolk entoderm. At about the end of the seventh or the beginning of the eighth day the first mesoderm appears at the posterior end of the future embryo between the external and internal cell layer of the egg cylinder (figure 4). Thus the wall of the egg cylinder consists of three layers at this place. In sections of the egg cylinder the division of the egg cylinder cavity into amnionic and ectoplacental cavities begins to become apparent (figure 5 and 6). Both of these cavities are still connected with each other at this stage.

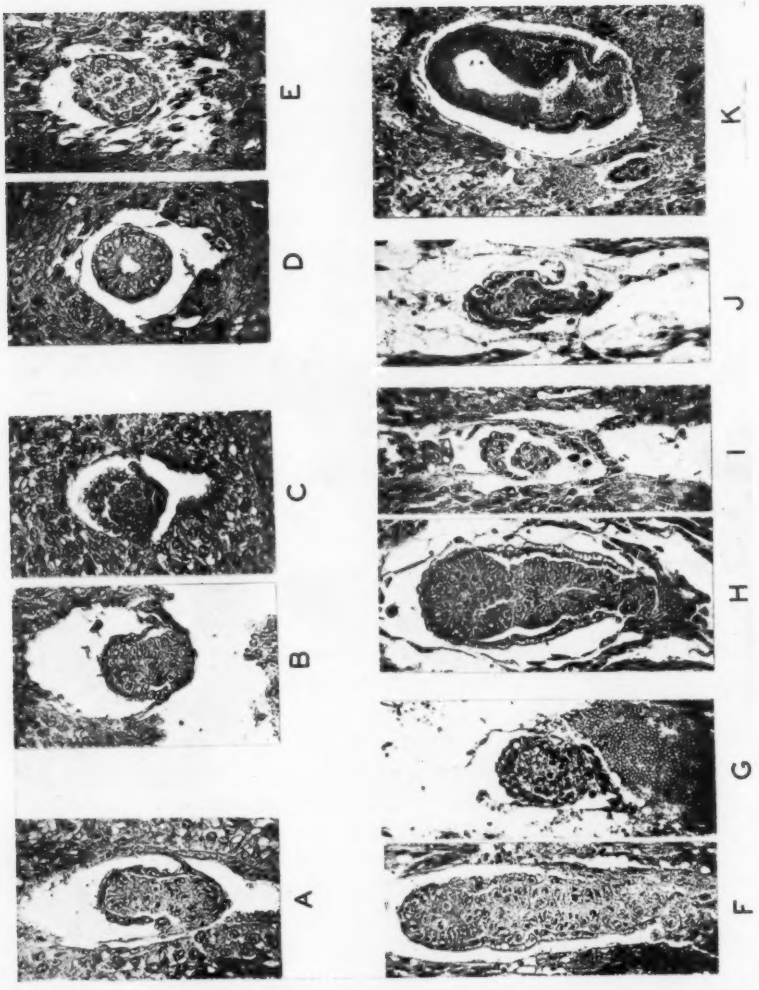
HISTOLOGICAL DESCRIPTION OF THE HOMOZYGOUS EMBRYOS (l^0l^0)

Among 32 embryos from three early litters (aged four days, 23 hours to five days, three hours) only three abnormal embryos were found. These three which were dead and in the process of being resorbed, had apparently died from accidental causes. At this early stage (the end of the fifth day) the embryo has just become implanted. Plate 1, figure A gives a typical picture of such a young embryo in the early egg cylinder stage. It has not been possible so far to detect any differences between normals and typical abnormals at this early stage. In a litter dissected five days five hours after copulation the earliest typical differences between normals and abnormals were observed. Figures B and C both show oblique sections through a normal (B) and an abnormal (C) embryo of this litter. There are two main differences to be observed between the two types. First, the normal embryo shows the typical differentiation of the ectoderm into two spherical cell masses while the abnormal embryo has an undifferentiated ectodermal center mass. Second, the entodermal layer of the normal embryo is thin and regular, while that of the abnormal embryo is thick and irregular; the individual entoderm cells of the abnormal embryo are larger and have more cytoplasm than the normal entoderm cells.

Figures D and E show the differences between normals and lethals to have become more obvious at a slightly older stage. Figure D represents a cross section through the egg cylinder of a normal embryo, five days 20 hours $\pm$ three hours after copulation. Figure E shows a cross section through its abnormal litter mate. The abnormal embryo is not dead yet, as the finding of a mitosis indicates; the difference from the normal can be found in the disorganization of the ectodermal cell mass (for example, the absence of the first trace of the proamnionic cavity) and in the disorganized appearance of the outer (entoderm) layer.

Figures F and G show two littermates—one normal, the other abnormal —in longitudinal sections. The normal embryo is in the stage of the elongated egg cylinder. Mesoderm formation has not occurred as yet. The abnormal embryo is much smaller than the normal and consists of a uniform inner cell mass which is surrounded by a heavy layer of very large entoderm cells. This picture reminds one of a very early normal stage (figure A) except for the thickness of the entoderm and the disorganization of the inner cell mass. It looks as if the embryo had stopped developing at the very early egg-cylinder stage and abnormal processes had then set in which resulted in thickening of the entoderm and disorganization of the ectodermal cell mass.

Figures H and I show longitudinal sections through embryos slightly older than those pictured in figures D and E, figure H being a section of a normal embryo in a stage just before the appearance of mesoderm, figure I


A
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that of an abnormal litter mate. This latter picture is quite typical of the abnormal type in this stage: there is an ectodermal cell mass with living cells in the center (presence of mitoses) and an entodermal cell cap consisting of many large entoderm cells with a great amount of cytoplasm. While in the stage preceding this, entoderm and ectoderm are still in close contact, these two layers have now separated. The entoderm "cap" has been lifted off the ectodermal cell mass. This phenomenon, although found frequently in this stage, is not regularly observed.

The development of the normal embryo proceeds at a rapid pace at this time. The egg cylinder elongates, and mesoderm appears; the amnionic folds begin to form and the formation of the proamnionic cavity, ectoplacental cavity and exocoelom proceeds.

While all these developments go on in the normal embryo within approximately 24 hours, the picture of the lethal embryo does not change very much. There is always an inner cell mass surrounded by a heavy entoderm layer with the entoderm cells being very large and proliferating most strongly at the antimesometral pole of the embryo (figure J). Sometimes this entodermal "cap" is lifted off as shown in figure I, but sometimes the outer entoderm stays in close contact with the inner cell mass. More and more degenerated and granulated cells are found in the abnormal embryo, until by the end of the seventh day nothing but a mass of pycnotic granules is left.

Figure K represents a curious phenomenon encountered in sections of one of the embryonic capsules, dissected at the age of seven days, three

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DESCRIPTION OF PLATE 1

Photographs of sections of mouse embryos showing effects of lethal t^6t^6.

FIGURE A: 4918,4l. Age 5 days 3 hours ± 1 hour. Longitudinal section through embryo in the young egg cylinder stage. 250×.

FIGURE B: 4907,3r. Age 5 days 5 hours ± 1 hour. Oblique section through normal embryo. 250×.

FIGURE C: 4907,2r. Age 5 days 5 hours ± 1 hour. Oblique section through abnormal embryo, litter mate of figure B. 250×.

FIGURE D: 4908,5r. Age 5 days 20 hours ± 3 hours. Cross section through normal embryo. 250×.

FIGURE E: 4908,6l. Age 5 days 20 hours ± 3 hours. Cross section through abnormal embryo, litter mate of figure D. 250×.

FIGURE F: 4108,3l. Age 6 days ± 1 hour. Longitudinal section through normal embryo. 250×.

FIGURE G: 4108,4r. Age 6 days ± 1 hour. Longitudinal section through abnormal embryo, litter mate of figure F. 250×.

FIGURE H: 4071,1l. Age 5 days 21 hours ± 1 hour. Longitudinal section through normal embryo. 250×.

FIGURE I: 4071,2l. Age 5 days 21 hours ± 1 hour. Longitudinal section through abnormal embryo, litter mate of figure H. 250×.

FIGURE J.: 4138,4l. Age 6 days 3 hours ± 3 hours. Longitudinal section through abnormal embryo. 250×.

FIGURE K: 4021,1l. Age 7 days 3 hours ± 4 hours. Longitudinal section through two embryos, implanted next to each other in utero, one normal (right), the other abnormal (left). 120×.

hours ± four hours. Two embryos, one normal, the other lethal, had become implanted next to each other in the uterus. Thus, the picture of a section through these two embryos presents an excellent demonstration of the differences between normal and lethal embryos. The difference in size is obvious; while the normal embryo shows the amnionic cavity, the amnionic folds and the appearance of mesoderm between the inner (ectodermal) and the outer (entodermal) layer, the lethal embryo shows the typical abnormal picture: an unorganized inner cell mass in which mitoses were found, with a heavy entoderm layer around it. Some cells at the top of the cylinder begin to disintegrate.

DISCUSSION

From the histological description of the development of the t^0t^0 embryo it appears that normal and lethal embryos cannot be distinguished before the age of five days. The very young egg cylinder stage is identical for both normals and lethals. But while the normal embryo soon begins to grow rapidly and morphogenesis proceeds, growth and development of the abnormal embryo cease. Until about the age of six and a half days, the lethal embryo is characterized by an unorganized inner cell mass surrounded by a heavy entoderm layer; the whole embryo has the shape of a young egg cylinder. This unorganized cell mass begins to show signs of degeneration around the age of six and a half days; it soon becomes necrotic and finally is resorbed.

The death of the embryos in the A line at about the age of seven days is due to their genetic constitution and not to accidental causes. A comparison of the data presented here with those obtained in the 29 line (GLUECKSOHN-SCHOENHEIMER 1938b), corroborates this conclusion. In the 29 line, in which death of t^1t^1 occurs before implantation, 21 litters dissected at the age of seven and eight days, contained 152 embryos of which 146 were normal and six (four percent) were abnormal. Dissections of 10 litters in the A line at the corresponding age yielded 101 embryos of which 60 were normal and 41 (40.6 percent) were abnormal. Since the A line and the 29 line have the same genetic background (Bagg albino) and differ chiefly in the genes t^0 and t^1, the great number of abnormal and dead embryos at the age of seven and eight days in the A line must be attributed to the lethal action of t^0t^0. The eight (2.9 percent) atypical abnormals found in the A line and mentioned above may be comparable to the four percent of abnormals found in the 29 line.

In the study of the development of the tailless mouse Tt^0 it was found that notochord, mesoderm and hindgut were affected in the embryo. These structures—according to SOBOTTA'S papers—can be traced back to the re-

gion of the primitive gut and it was suggested that a malformation in the region of the primitive gut might give rise to the malformations described for the tailless mouse. The fact that the homozygous lethal t^0t^0 stops its development just before the time of primitive gut and mesoderm formation suggests that a fundamental disturbance in organization occurs in this abnormal embryo. Perhaps one might be permitted to call this disturbance a complete failure of gastrulation, gastrulation in the mouse being the process of formation of primitive gut, primitive streak and mesoderm.

CHESLEY was able to show that T has an effect primarily on the notochord. From the present study it appears that t^0 may act primarily on the mesoderm.

One may attempt to correlate in the following manner the different malformations and their development in the different genotypes carrying T and t^0. In the heterozygous Brachy ($T+$) mouse the notochord in the posterior region of the embryo is defective and as a result the Brachy phenotype develops. In the tailless mouse Tt^0, the notochord and the mesoderm of the posterior region of the embryo are defective as a result of which the phenotype of the tailless mouse develops. In the homozygous Brachy mouse (TT) the entire notochord is affected; this embryo dies on the 11th day of development. The t^0t^0 homozygote—described in this paper—fails to form any mesoderm; the embryo dies on the seventh day of development, that is, soon after the beginning of mesoderm formation in the normal. It is impossible to decide whether the lack of mesoderm formation and of further differentiation is the cause of death, or whether the failure to form mesoderm and to differentiate are secondary to some abnormal condition of the embryo which eventually leads to its death. In the latter case the lack of mesoderm and the failure to differentiate would be only two phenomena of the entire syndrome. Only evidence from transplantation experiments could reveal the role that the mesoderm (or the lack of mesoderm) plays in the development of the abnormal embryo.

The effect of the two alleles T and t^0 on notochord and mesoderm might suggest that the two alleles act on two *different* structures. However, if considered from the embryological point of view, the notochord and mesoderm of the mouse have the same origin, namely in the tissue of the wall of the primitive gut. Since the t^0t^0 homozygote does not live until the time of notochord formation, it is not possible to decide whether it would be able to form notochord; the complete failure of formation of mesoderm therefore excludes the possibility of notochord formation.

KOBOZIEFF and POMRIASKINSKY-KOBOZIEFF, in a recent paper (1939) report that dissections of uteri of pregnant females from tailless by tailless matings gave two classes of lethals, and dissections from $+t^0$ by $+t^0$ mat-

ings, one class of lethals. This agrees with our findings. The authors do not mention the time of death nor any detailed examination of the abnormal embryos.

SUMMARY

The development and time of death of the t^0t^0 homozygote are described.

The t^0t^0 homozygote can first be distinguished from its normal littermates at the age of about $5\frac{1}{4}$ days after fertilization.

At this age the development of the homozygote stops. It remains alive for about 40 hours, without showing any signs of growth, organization or mesoderm formation.

At about seven days after fertilization necrosis of the homozygous embryo sets in and resorption takes place.

From the study of the development of Tt^0 and t^0t^0 it is concluded that t^0 affects development and formation of mesoderm.

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CYTOGENETIC STUDIES ON TRITICALE. I. A METHOD FOR DETERMINING THE EFFECTS OF INDIVIDUAL SECALE CHROMOSOMES ON TRITICUM[1]

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THE production of fertile, true-breeding hybrids which combine the characters of two different species or genera has proven to be of theoretical and practical importance. These hybrids have usually arisen through a doubling of the chromosome complement following the initial cross, this doubling resulting in the presence in the fertile hybrid of two full complements from each parent. Such plants are known as amphidiploids because the chromosome set of each of the two parents is present twice, or in the diploid condition. It is possible, however, to combine characters of two species or genera by a method which involves the addition of one or more chromosomes from one species to the full diploid complement of the other species. When the chromosomes of one species have desirable and undesirable dominant characters distributed among the various chromosomes of the set, these characters are given to the second species without selection in the amphidiploid. If only the chromosomes with the desired dominant characters could be added to the chromosome complement of the second species, the value of the combination for theoretical and practical purposes would be greatly enhanced. Such combinations have been secured between Secale and Triticum, and the method by which they were obtained should be applicable to other genera where an amphidiploid is available.

The method, in brief, involves the production of an amphidiploid between two species or genera, A and B. The amphidiploid is then crossed to one of the parents—for illustration, species A. The individual obtained from this latter cross would have two full sets of chromosomes from species A and one full set from species B. During meiosis, the two sets from species A would undergo normal pairing and distribution, whereas the set from species B would remain as univalents and would be distributed at random. Thus the gametes from such a plant would contain a full set of chromosomes from species A, and from none to a full set from species B. From such a plant the offspring would contain two full sets of chromosomes from species A and from none to various combinations of the chromosomes of species B.

[1] These studies are a part of the program supported by funds obtained under Bankhead-Jones Project SRF-2-5, "Comparative Genetics and Cytology of Polyploidy Series in Triticum," Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and Missouri Agricultural Experiment Station, cooperating.

CHROMOSOME BEHAVIOR OF THE PARENTAL TYPES

The amphidiploid used in these experiments (produced by E. R. SEARS) consisted of spring rye, *Secale cereale*, and "Chinese" spring wheat, *Triticum vulgare*, and was derived directly from the first generation hybrid by duplication of the chromosomes in somatic tissue. These three types may be seen in figure 1, where a is the rye, c the wheat, and b the amphidiploid. The wheat-rye hybrid has been studied cytologically by KIHARA (1924), THOMPSON (1926), LONGLEY and SANDO (1930), FLORELL (1931), and KATTERMANN (1934). KIHARA reported that the hybrid, at metaphase 1, had from one to three bivalents, and his report was confirmed by THOMPSON, who found the same numbers of bivalents, and who further reported that he believed the bivalents to be the result of autosyndesis among the wheat chromosomes. LONGLEY and SANDO observed no pairing in this cross but found the same number of bivalents, from one to three, in a hybrid involving *Secale montanum*. FLORELL observed that in the wheat-rye hybrid, involving Hybrid 128 wheat and Rosen rye, from one to three bivalents were formed, approximately half the cells having two bivalents. KATTERMANN reported as many as six bivalents in some cells. The amphidiploid between wheat and rye has been made several times, and its cytology has been most thoroughly investigated by MÜNTZING (1939). All the amphidiploids reported thus far have been asynaptic to some degree, univalents occurring at metaphase 1. The amphidiploid used in these experiments had approximately 43 percent of the cells without univalents. No Triticale strain with a perfectly regular meiosis has been reported. As a result of this irregularity, variations occur in the chromosome numbers of the gametes and of the progenies.

For the purposes of the present method of procedure, the production of a plant with two full complements of wheat chromosomes and one full complement of rye chromosomes is absolutely necessary. An amphidiploid sector on a wheat-rye hybrid was backcrossed to wheat. A single plant from this cross was examined cytologically and found to have 21 normally-behaving pairs of wheat chromosomes and seven univalent rye chromosomes. This plant was allowed to self, and from it 70 seeds were obtained.

THE PROGENY OF THE PLANT DIPLOID FOR WHEAT AND HAPLOID FOR RYE CHROMOSOME COMPLEMENTS

The 70 seeds obtained from the plant which had two wheat complements and one rye complement produced 70 plants. These plants had 21 pairs of wheat chromosomes and various numbers of rye chromosomes, ranging from one to seven. The presence of rye chromosomes in these plants produced very striking effects on the morphology of the plants. All the rye chromosomes occurred as univalents, which were distributed in the random fashion usual for univalents.

One plant among the 70 is interesting in that it had 28 univalent chromosomes at the first meiotic division. Presumably, this plant resulted from the parthenogenetic development of an egg which had received the full set of wheat chromosomes plus all seven of the rye chromosomes. This plant, then, should have the same constitution as a first-generation wheat-rye hybrid, since it had a haploid set of wheat and a haploid set of rye chromosomes.

Twenty-nine of the 70 plants were selected for carrying on the rye chromosomes to the next generation. These plants were quite fertile, possessed certain rye characters, and showed 21 pairs of normally-behaving wheat chromosomes plus one or two rye univalents at metaphase 1. The plants were selfed by bagging the heads before anthesis. From the 29 progenies of these plants, a single plant which carried the character under consideration, was selected from each of 18 progenies, and these 18 plants were selfed. The selfing was carried on, in this generation, to reduce the number of rye chromosomes to one. Since the rye chromosomes were present as univalents, they tended to be lost at meiosis for two reasons: (1) univalents are often lost in the cytoplasm at meiosis, where they form micronuclei; (2) each univalent is distributed to but half the gametes, and so the number decreases in successive generations. These 18 plants consisted of nine plants which had hairy-neck, two which had tapered heads, and seven which had awns. These characters were selected for study because their distribution among the 70 plants indicated that they were in different chromosomes. The progenies of the 18 plants were examined cytologically.

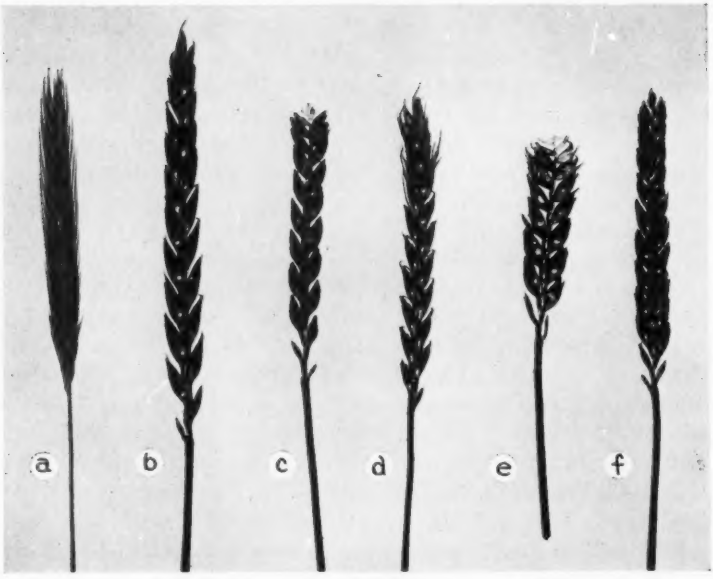
In the progenies of eight of the nine hairy-neck plants, approximately one third of the individuals had hairy-neck. In the progeny of the remaining hairy-neck plant, consisting of 19 individuals, all the plants had hairy-neck. Upon examination, all these 19 individuals proved to have at least 22 pairs of chromosomes, that is, 21 wheat bivalents plus one rye bivalent. Three of the 19 plants had 23 pairs of chromosomes. In the progenies which were segregating for hairy-neck, all but two of the affected plants had the rye chromosome present as a univalent. These two plants had 22 pairs of chromosomes. They were disomic for the rye chromosome and similar in appearance to the plants of the progeny which had all 22-chromosome plants. The rye chromosome, when disomic, did not always form a bivalent but was present in a small percentage of cells as two univalents, and when present as a bivalent, often separated when the wheat chromosomes were at anaphase.

The hairy-neck chromosome had a marked effect upon the plant, other than the characteristic pubescence just below the head. Since all the plants which had the character were nearly identical in appearance, it is very probable that but one of the rye chromosomes carries the factor or factors involved in producing the character. Those plants which were monosomic

for this chromosome were slightly shorter than normal wheat plants and had heads with broader, coarser spikelets. When the chromosome was disomic, these alterations were exaggerated, and the plants were approximately two thirds the height of normal wheat, and had coarser, broader spikelets than the plants monosomic for the chromosome. These differences may be seen by comparing c, a normal head, with e, a head disomic for the hairy-neck chromosome, in figure 1. A comparison of the amounts of pubescence on the necks of plants monosomic and disomic for the rye chromosome and on the neck of the amphidiploid is interesting, in that such a comparison shows that the amphidiploid has the least pubescence, although it has the rye chromosome present in two doses. This comparison would seem to indicate that no accurate predictions of the degree of manifestation of any character can be made from the amphidiploid. The derived type may be more like one parent than like the amphidiploid, although both have identical doses of the chromosome concerned.

The rye chromosome which carries the factors or factor producing tapered head, imparts to the individual possessing it a head without any compacting at the tip, such as is characteristic of the wheat parent. The two progenies having this character were found to be different, one retaining several extra rye chromosomes. This one was not studied. The other progeny consisted of 30 plants, five of which had the character. Four of these five were monosomic for the chromosome, and the other plant was disomic for it. This rye bivalent was less stable than the hairy-neck bivalent, occurring in many cells as two univalents. The univalents can always be identified as rye chromosomes—and not wheat—by their larger size and consistent form. The effect of this chromosome on the plant, other than that of producing the tapered head, was to increase the height of the plant so that individuals carrying the chromosome as a monosome were slightly taller than normal wheat plants, and the plant which carried the chromosome as a disome was over a third taller. The effect of this chromosome, therefore, seemed to be additive, since the disomic was taller than the monosomic plant. The difference, however, was not so marked as with the hairy-neck chromosome.

The term "tapered" was applied to this character because the heads of the affected plants did not have the compacted tip characteristic of the parental wheat, but tapered continuously from basal to apical spikelets. A comparison of head c—which is normal—and f, which carries the chromosome as a disome, will show the difference. At least one other rye chromosome, however, has a similar effect, and the term was used for this chromosome because no clearly separable effect, other than "tapered," was detectable, whereas another chromosome which produces the same effect carries, in addition, a factor for producing awns.



DESCRIPTION OF FIGURE 1

Heads of plants of various chromosome constitutions. ($\times\frac{2}{3}$)

a. *Secale cereale* involved in amphidiploid illustrated at b.
b. Amphidiploid involving a, *Secale cereale*, and c, *Triticum vulgare* var. "Chinese spring."
c. *Triticum vulgare* var. "Chinese spring" involved in amphidiploid illustrated at b.
d. Triticum to which has been added the Secale chromosome producing awns. The chromosome is present as a disome.
e. Triticum to which has been added the Secale chromosome producing hairy-neck. The chromosome is present as a disome.
f. Triticum to which has been added the Secale chromosome producing tapered-head. The chromosome is present as a disome.

The seven progenies from awned plants did not fall into distinct classes; five were segregating, with approximately one third of the individuals awned, one had seven awned plants and two normal wheat plants; and one progeny consisting of 13 individuals had all awned plants. The segregating progenies had an extra univalent chromosome, and the group with all awned plants had an extra bivalent. This bivalent seemed to be fairly regular in behavior, and although it sometimes separated late, most cells at anaphase 1 had 22 chromosomes at each anaphase pole.

The chromosome bearing the factor or factors for awns had the most striking effect on the plant, especially when disomic. The plant is usually shorter than normal wheat, has no compacting of spikelets at the tip, has many fine, dark green leaves when young, is inclined to be spreading in habit, and possesses awns approximately one fifth the length of those of the rye parent. The awns are not uniform throughout the head, being nearly absent from some spikelets, and generally longest at the tip of the head, as may be seen in d of figure 1.

In one segregating progeny a plant was discovered which had 23 bivalents and one univalent. This plant had both awns and hairy-neck, so that two of the three chromosomes represented may be accounted for by phenotypic effects. No effect of the other chromosome or chromosome pair was observable.

In addition to the progeny of the single plant secured by backcrossing the amphidiploid to the wheat parent, the progeny of one other plant of similar parentage was grown. Of this progeny seven plants were examined cytologically. One of these had 22 pairs of chromosomes plus two univalents,—an instance which indicates that the bivalents may be added directly in the progeny of the backcrossed plant, although none of the original 70 plants had such an extra bivalent.

THE INTRODUCTION OF RYE BIVALENTS

Thus far all attempts to secure rye characters in wheat-like plants have been made by using the F_1 hybrids in backcrosses to wheat. This method has, in some respects, proved quite practicable, as the results of LEIGHTY and TAYLOR (1924), of FLORELL (1931), of TAYLOR (1939), of KATTERMANN (1934), (1935), (1937), (1938), and of LEDINGHAM and THOMPSON (1938) indicate. Any additions to the basic wheat complement obtained by this method may, however, consist of wheat chromosomes or chromosome pairs and not of rye, as LEDINGHAM and THOMPSON concluded from their studies on progenies of wheat-rye hybrids backcrossed to wheat. KATTERMANN'S extensive studies on plants secured by this method of using the F_1 hybrid indicate that the female gametes of the hybrid may have from 17 to 31 chromosomes. While variations occur in the gametes of the am-

phidiploid, none of this order are indicated by the data of MÜNTZING (1939). The hybrid, from a cytological viewpoint, at least, is comparable to a haploid; and SEARS' (1939) studies on the progeny of a haploid wheat indicate that duplications of whole chromosomes occur in the female gametes. These duplications in the hybrid should involve wheat chromosomes three times as often as rye. Further, the haploid was found by SEARS to produce very aberrant progeny—monosomes, trisomes, and segmental interchanges—all of which would usually be undesirable where the process of addition is to be carefully controlled.

The origin of the present wheat plants disomic for a rye chromosome is capable of two interpretations. Since none of the 70 original plants—secured from the plant with two wheat complements and one rye complement—had pairs of rye chromosomes, these must have occurred in a later generation. That the chromosome is not usually transmitted through the pollen may readily be concluded, since in segregating progenies disomic plants are rare; and no pairs were found among the 70 plants where a maximum probability exists for finding pairs. These disomic plants, however, may occasionally occur as the result of a monosomic pollen grain competing successfully with normal pollen. The other possibility is that in a plant with a single rye chromosome non-disjunction—or some other meiotic anomaly—may occur to produce a disomic egg which, when fertilized by a normal pollen grain, would produce a disomic plant. Either of these methods is possible, and each may be tested by appropriate backcrosses to normal gametes of eggs and pollen from monosomic plants.

Should these disomic plants prove not to be true-breeding—and so resemble those of LEDINGHAM and THOMPSON—they should nevertheless, by chance distribution, make available monosomic and disomic plants in appreciable numbers in succeeding generations.

Another method could be used to secure these results. If the plants with rye univalents were pollinated by the amphidiploid, each univalent would be supplied with its homologue. Those chromosomes without homologues would be eliminated by successive selfings.

The failure of the rye chromosomes to pair as regularly as the wheat chromosomes is difficult to explain. It may represent some inability of rye to operate normally in a cell which is almost wholly wheat. The tendency to asynapsis may also be due to the absolute homozygosity of the rye chromosome and the consequent uncovering of recessive factors which have deleterious effects on the regularity of meiosis. However, all such factors now known affect meiosis as a whole and not merely the single chromosome which bears them.

USES OF THE DERIVED TYPES

These plants with single chromosomes from one species added to the full unaltered chromosome complement of a wholly different species, have several obvious practical and theoretical uses. They could be used to determine efficiently what factors dominant to the genome upon which they were superposed were linked together in the single chromosome. By crossing two plants homozygous for different chromosomes, the interaction of these two known chromosomes could be studied. By using the plants in which single chromosomes had been isolated, various tests for disease resistance, hardiness, and various other factors important economically, could be made to determine if the trait desired were located in a single chromosome or were a property of the whole complement. Such information should indicate whether any single property desirable in one genome could be transferred to the other through rare non-homologous pairing or through induced translocations. Such useful transfer is obviously impracticable where the desirable character is a property of the whole genome or of many factors located in different chromosomes.

ACKNOWLEDGMENT

The author is indebted to DR. E. R. SEARS, who furnished the original seed of the amphidiploid and its backcross which were used in these experiments, and to Dr. L. J. STADLER and DR. BARBARA MCCLINTOCK for suggestions during the preparation of the manuscript.

SUMMARY

By backcrossing an amphidiploid to one of its constituent species and selfing for several generations, individuals were produced with a single chromosome pair from one species in the amphidiploid added to the unaltered chromosome complement of the other species. These additions represent three different rye chromosomes, phenotypically detectable, which have been added to an unaltered wheat genome. The method should be applicable to other genera where an amphidiploid is available and may have some value in practical and theoretical genetics.

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THE GENETICS OF SEX DETERMINATION IN THE FAMILY AMARANTHACEAE*

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INTRODUCTION

AS HAS been emphasized by YAMPOLSKY (1922, 1925) angiospermous plants exhibit many physiological as well as morphological forms of sex expression. Many of these forms appear to be intermediate steps in the evolution of one type into that of another, as has been suggested by CORRENS (1928) and others. EMERSON (1924), JONES (1934), and LEBEDEFF (1938) have predicted that the many gene mutations which affect sex in maize are the building blocks that could lead to the development of a different type of sex expression in this plant. In fact, EMERSON (1932) and JONES (1932, 1934) have produced dioecious strains of maize by the proper combinations of two genes.

Is the evolution of dioecious forms from hermaphroditic ones, or the reverse, explainable on such a simple genetic basis? CORRENS (1907, 1928) early attempted to solve this question by direct hybridization, but the sterility of his F_1 hybrids did not allow him to continue his analysis beyond the first generation. Recent work on cultivated species and varieties indicates that sex expression is markedly influenced by a few gene mutants having very specific actions. STOREY (1938 a, b) and HOFMEYR (1938) believe that a single gene with two alleles will explain much of the sex variability within the species *Carica Papaya*. OBERLE (1938) found that variations in the sex expression of Vitis species, varieties, and hybrids is best explained on the basis of two allelic pairs of factors which are completely linked. ROSA (1928) has shown that a monoecious condition in muskmelons is dominant to an andromonoecious condition and that this difference is due to a single factor. POOLE and GRIMBALL (1939) have shown that monoecious and perfect-flowered varieties of muskmelons differ in two gene mutations and that the perfect-flowered form is genetically the double recessive type. Do these same conclusions apply to natural species which have evolved two different but stable sex forms?

The work reported in this paper represents a search for suitable material which would allow a direct genetic analysis of second generation progeny

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resulting from the hybridization of dioecious and hermaphroditic forms. Members of the family Amaranthaceae were selected at PROFESSOR R. A. EMERSON'S suggestion, since he had observed in nature sterile F_1 hybrids arising from the natural hybridization of a monoecious and a dioecious species. As a large number of closely related monoecious and dioecious species are available in this group of plants, certain combinations might be found which would give fertile hybrids, unless there is some direct relation between sterility and the evolution of sex differences. A detailed account of this work is given here. Preliminary reports in abstract form have already been published (MURRAY 1938, 1939, 1940 a, b).

MATERIALS AND METHODS

Species which have been used successfully in crosses are given in table 1. Numerous pollinations were also made between this material and species in other genera such as Celosia, Gomphrena and Achyranthes but no hybrids were obtained.

TABLE 1

Origin, sex, and chromosome number of the species used in this study.

SPECIES	ORIGIN	SEX	N CHROMOSOME NUMBER
Amaranthus hybridus L. Angol race	Near Angol, Chile	Monoecious type 1	16
Amaranthus hybridus Line 58	Ithaca, N. Y.	Monoecious type 1	16
Amaranthus hybridus Line 56	Ithaca, N. Y.	Monoecious type 1	16
Amaranthus caudatus L. var. leucospermus (Wats.) Thell. Mexican race	Mexico	Monoecious type 1	16
Amaranthus caudatus L. var. leucospermus (Wats.) Thell. Manchurian race	Manchuria	Monoecious type 1	16
Amaranthus retroflexus L.	Ithaca, N. Y.	Monoecious type 1	17
Amaranthus Powellii Wats.	Kansas		
	Ithaca, N. Y.	Monoecious type 1	?
Amaranthus spinosus L.	Central Yucatan	Monoecious type 2	17
	Belle Glade, Fla.		
Acnida tamariscina (Nutt.) Wood	Lincoln, Neb.	Dioecious	16
Acnida cuspidata Bert.	Canal Point, Fla.	Dioecious	16
Acnida tuberculata Moq.	Westerville, Ohio	Dioecious	16

The material was identified by DR. P. C. STANDLEY of the Field Museum of Natural History who has monographed this family. It is well known by taxonomists that this is, at least in part, a difficult group and that the correct application of names in some cases requires study of type specimens present in European herbaria. To know the exact material used in this study, reference may be made to specimens deposited in the Cornell University herbarium or the Field Museum of Natural History, Chicago.

The three races of *Amaranthus hybridus* have been treated as though they were species since they are morphologically distinct in habit, leaves and inflorescence characters. They breed true to type and at least part of their hybrid combinations are almost completely sterile.

While taxonomic manuals disagree in their descriptions of the sex of the several members of the genus Amaranthus, the races and species which I have used are strictly monoecious. The arrangement of the staminate and pistillate flowers within the inflorescence is a very definite one. This may be seen from a consideration of the growth pattern shown by the species used in this work The main axis of the inflorescence is usually branched.

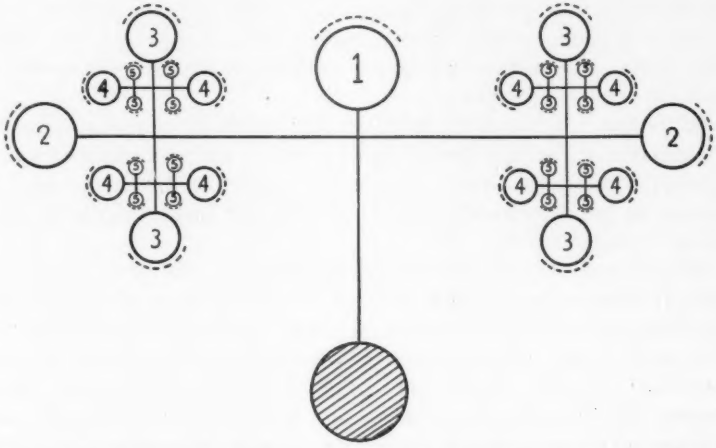

FIGURE 1.—Diagrammatic illustration of the arrangement and development of individual flowers within a flower cluster. A broken line indicates the position of the bract which subtends each flower. The shaded circle is the main axis of the inflorescence.

The length and number of these branches and their angle with the main axis determine the shape of the inflorescence. Individual flower clusters develop along these axes in an alternate fashion, while within each flower cluster, individual flowers are produced as illustrated diagrammatically in figure 1. The first flower is terminal on the branch and at its base, two branches develop the second and third flowers. Each of these flowers in turn is terminal and at its base develop the next two flowers. This process continues until all the available space is occupied. Development is usually very symmetrical up to the third or fourth series of flowers. At this time the setting of the first seed usually slows down growth and upsets the symmetry. Unpollinated clusters may develop as many as 250 flowers. My diagram has purposely exaggerated distances between the flowers and has also

placed the branches exactly opposite each other as they appear to be in gross dissections. The apparently opposite branching in the flower cluster is probably produced by shortening the axes of an alternate branching system.

This growth pattern is common to all the species regardless of sex. In the dioecious species, the flower clusters not only have all their flowers of the same sex but all the flower clusters on one plant are of the same sex. The monoecious species exhibit two types of arrangement of the staminate and pistillate flowers. These types are important because of their different breeding behavior.

In the first type, the first flower of each flower cluster is staminate and all the succeeding ones are pistillate. There is only one staminate flower in each flower cluster of the inflorescence and this abscisses soon after shedding pollen. The monoecious species of Amaranthus except *Amaranthus spinosus* belong to this group.

In the second type, all the flowers of each cluster are of the same sex but the clusters of pistillate flowers develop only in the axils of the branches and at the base of the terminal inflorescence while the clusters of staminate flowers are borne terminally on the main axis and lateral branches. *Amaranthus spinosus* is of this type.

All species used in this study are wind-pollinated. The monoecious members are chiefly self-pollinated, although the stigmas of the pistillate flowers are receptive several days prior to the opening of any staminate flowers. The small, closely grouped flowers of the monoecious species made emasculation extremely difficult. The most satisfactory method of making crosses onto the monoecious species is to pollinate heavily as soon as the stigmas are receptive and to remove the staminate flowers by hand. Even so, 5–25 percent self-pollination usually occurs. The hybrids are easily distinguished from the monoecious parent and in several crosses dominant genes have been used to distinguish the hybrids in the seedling stage.

Six kinds of crosses have been made and will be discussed in the order given below.

1. Interspecific crosses of dioecious species of Acnida.
2. Interspecific crosses of monoecious species of Amaranthus.
3. Intergeneric crosses of dioecious species of Acnida and monoecious "first type" species of Amaranthus.
4. Intergeneric crosses of dioecious species of Acnida and monoecious "second type" species of Amaranthus.
5. Backcross of F_1 hybrids obtained from "3" to both parental types.
6. Backcross of F_1 colchicine-induced tetraploid hybrids obtained from "3" to both parental types.

RESULTS

Interspecific crosses of dioecious species of Acnida

Three different dioecious species breed true to type and give normal 1:1 sex ratios (table 2). All six possible F_1 hybrids have been made and each of these gives a 1:1 sex ratio, with the possible exception of the first cross which shows a deviation slightly greater than should be expected by chance.

TABLE 2

Hybridization results of interspecific crosses of dioecious species of Acnida.

CROSS	FEMALE	MALE	D./P.E.	
			1:1	1:2
A. Control—Dioecious species inbred:				
Acnida tamariscina (Nutt.) Wood	2259	2291	.75	
Acnida cuspidata Bert.	72	64	1.02	
Acnida tuberculata Moq.	43	39	.66	
B. First generation progeny of interspecific crosses:				
A. tamariscina×*A. cuspidata*	752	664	3.47	
Reciprocal	622	568	2.32	
A. tamariscina×*A. tuberculata*	83	100	1.97	
Reciprocal	58	62	.54	
A. cuspidata×*A. tuberculata*	173	179	.47	
Reciprocal	80	102	2.42	
C. Second generation progeny of interspecific crosses:				
F_2 (*A. tamariscina*×*A. cuspidata*)	196	167	2.3	
B.C. (*A. tamariscina*×*A. cuspidata*)× *A. tamariscina*	67	137		.22
F_2 (*A. cuspidata*×*A. tamariscina*)	73	79	.72	
B.C. (*A. cuspidata*×*A. tamariscina*)× *A. tamariscina*	71	84	1.66	
B.C. (*A. cuspidata*×*A. tamariscina*)× *A. tamariscina*	49	116		1.46

The second generation progeny consist of normal male and female plants. Certain crosses give 1:1 sex ratios while other crosses give ratios of one female to two males. The latter type of cross is due apparently to the presence of a recessive sex-linked lethal carried in the X chromosome. If this is true, the genotypes of the F_1 parents are X^lX^+ and X^lY^+. The male is known to be the heterogametic sex from genetic evidence presented later in this paper.

No intersexes, hermaphroditic plants, or sex abnormalities were found in the second generation progeny obtained from reciprocal hybridization of these two different dioecious species. Why should this be true? If individual chromosomes of these two species carry different quantitative amounts of

male and female determining factors (due either to the quantitative nature of a few genes or to the number of these genes), why does not the normal distribution of the chromosomes in the F_1 hybrids result in the production of plants with an abnormal balance of sex factors? The segregation of two monofactorial differences furnishes genetic evidence that at least one or two chromosomes segregate in a normal manner and there is no evidence suggesting that other members behave differently. Two hypotheses to explain the above data are cited.

First, if sex is determined by a balance between many sex determiners which act in either a male or a female direction, we must almost certainly conclude that these two species have, chromosome for chromosome, an identical balance of these factors. This assumption seems unreasonable from an evolutionary viewpoint. Two species which differ primarily in quantitative characters probably have had genic changes in every chromosome (MURRAY 1940a). Major changes in the chromosome arrangement of the genic material have apparently occurred. Why should genes affecting size and shape of morphological structures mutate while genes influencing sex do not? If a primitive dioecious Acnida plant came originally from a hermaphroditic ancestor, we could postulate a mass mutation phenomenon to account for this fact. The further assumption follows that once this balance of many factors is created, stability of all or most of the sex factors results, even though mutation of other types continues. The dioecious and monoecious species of this group of plants are certainly closely related; otherwise one could hardly account for the 33 different intergeneric hybrids that have been obtained. The explanation just attempted becomes very complicated and may be disregarded as a much simpler hypothesis can explain the same data.

A few gene mutations of a very specific nature could occur and cause the evolution of a dioecious form from a hermaphroditic one. EMERSON (1932) and JONES (1932, 1934) have shown that this can be accomplished experimentally in maize, using only two mutants which have occurred spontaneously. Their examples have one mutant gene homozygous in both sexes; the second gene is homozygous in one sex and heterozygous in the other. These two factors are located on different chromosomes. The first gene may be termed an autosomal factor; the second gene creates the XX and XY mechanism. If all monoecious maize were suddenly exterminated and these dioecious races were saved, one would not be able to detect the presence of the autosomal gene. One might discover that one sex was homogametic and the other heterogametic.

The same reasoning may be applied to the sex mechanism in Acnida species. The autosomes either carry no genes influencing sex or carry them in a homozygous condition. If autosomal sex genes are present, they are prob-

ably few in number, since two dioecious species do not differ in this regard although they exhibit other major changes of chromosome structure and genic content. This discussion is concerned only with those factors which differentiate between hermaphroditic species and closely related dioecious species. There is abundant evidence in plants and animals that many loci on most or all of the chromosomes are concerned with the physiology and morphology of reproductive structures, but only those genes which are different in the two sex forms need enter into this discussion.

SCHAFFNER (1923) observed *Acnida tamariscina* and found that the sexual states of this species were extremely stable. I, too, have found that this species exhibits very few sex abnormalities, even under the extreme conditions of low light intensity and low temperature. Occasionally a staminate plant will produce a few flowers having a partially developed ovary. These more or less perfect flowers do not produce seeds as they wither and absciss soon after the pollen is shed. No abnormalities have been observed on pistillate plants.

Although the reversion of 101 females and 243 males to the vegetative state was observed, only one example of complete sex reversal has been found. This plant originally had about 25 male flowers. After growing vegetatively, the plant was entirely female and normal in every respect. Crosses made with this plant as a female gave 400 ♀ ♀ : 385 ♂ ♂ which shows that the plant had a female genotype even though a few male flowers were produced at one time.

Interspecific crosses of monoecious species of Amaranthus

All monoecious species and races breed true and have been inbred one to three generations before being used in crosses. The two types of arrangement of the staminate and pistillate flowers found in these monoecious species (table 1) are phenotypically very stable. Abnormalities are rarely encountered.

Thirteen crosses have been made between members possessing the first type of sex arrangement (table 3) and the hybrids, thus obtained, have the same sex arrangement. This result would be expected if all these species are homozygous for the factors controlling this particular positional placement of the staminate and pistillate flowers. These species hybridize readily but the F_1 hybrids are so highly sterile that F_2 plants are seldom obtained. The few that were secured did not show segregation. On the other hand, the high sterility makes it easy to get amphidiploid progeny following colchicine treatment of the growing point. When the treatment is effective, an abundance of seed is produced; otherwise, only a few seeds would be procured from an entire inflorescence. The five amphidiploids have the same sex arrangement as their parents.

TABLE 3

Hybridization results of interspecific crosses of monoecious species of Amaranthus.

CROSS OR SELF	MONOECIOUS	REMARKS
A. Control—Monoecious Amaranthus species selfed	Breed true to type	
	(Number of plants)	
B. Interspecific crosses of monoecious species:		
First type×first type:		
A. hybridus Angol race×*A. caudatus* Manchurian race	548	1st type
Reciprocal	119	1st type
A. hybridus Angol race×*A. caudatus* Mexican race	28	1st type
A. hybridus Angol race×*A. hybridus* Line 58	166	1st type
A. hybridus Angol race×*A. hybridus* Line 56	169	1st type
Reciprocal	71	1st type
A. hybridus Angol race×*A. Powellii*	124	1st type
A. hybridus Angol race×*A. retroflexus*	434	1st type
Reciprocal	363	1st type
A. hybridus Line 56×*A. Powellii*	1	1st type
Reciprocal	1	1st type
A. hybridus Line 58×*A. Powellii*	5	1st type
Reciprocal	15	1st type
A. hybridus Line 58×*A. retroflexus*	1	1st type
A. retroflexus×*A. Powellii*	9	1st type
Reciprocal	1	1st type
A. caudatus Mexican race×*A. retroflexus*	115	1st type
Reciprocal	5	1st type
A. caudatus Mexican race×*A. Powellii*	61	1st type
A. caudatus Manchurian race×*A. hybridus* Line 58	172	1st type
First type×second type:		
A. caudatus Manchurian race×*A. spinosus*	2	1st type
A. retroflexus×*A. spinosus*	2	1st type
A. hybridus Angol race×*A. spinosus*	20	Intermediate
C. F_2 progeny of interspecific crosses of monoecious species:		
F_2 (*A. retroflexus*×*A. caudatus* Mexican race)	3	1st type
F_2 (*A. retroflexus*×*A. spinosus*)	10	2nd type
D. Amphidiploid progeny of interspecific crosses of monoecious species:		
F_2 (*A. hybridus* Angol race×*A. retroflexus*)	71	1st type
F_2 (*A. caudatus* Mexican race×*A. retroflexus*)	106	1st type
F_2 (*A. caudatus* Manchurian race×*A. hybridus* Line 58)	242	1st type
F_2 (*A. caudatus* Manchurian race×*A. hybridus* Angol race)	49	1st type
F_2 (*A. hydridus* Angol race×*A. hybridus* Line 58)	16	1st type

Members of the two types of sex arrangement hybridize with great difficulty. The plants that have been obtained are certainly hybrids as they show two dominant leaf color genes carried by the male parent. The first type is completely epistatic to the second type when the species *A. caudatus* and *A. retroflexus* are crossed to *A. spinosus*. An intermediate condition

TABLE 4

F_1 hybridization results of intergeneric crosses of dioecious species of Acnida and monoecious "first type" species of Amaranthus.

CROSS	♀♀	♂♂	D./P.E. 1:1
Acnida cuspidata×*Amaranthus retroflexus*	8	0	
Reciprocal	287	282	0.37
A. cuspidata×*A. hybridus* Angol race	717	0	
Reciprocal	453	411	2.31
A. cuspidata×*A. hybridus* Line 58	53	0	
Reciprocal	133	113	1.89
A. cuspidata×*A. caudatus* Mexican race	124	0	
Reciprocal	53	48	0.89
A. cuspidata×*A. caudatus* Manchurian race	73	0	
Reciprocal	144	121	2.19
A. cuspidata×*A. Powellii*	40	0	
Reciprocal	—*	—	
Acnida tuberculata×*Amaranthus retroflexus*	—	—	
Reciprocal	82	92	1.12
A. tuberculata×*A. hybridus* Angol race	346	0	
Reciprocal	276	314	2.32
A. tuberculata×*A. hybridus* Line 58	—	—	
Reciprocal	87	98	1.31
A. tuberculata×*A. caudatus* Mexican race	—	—	
Reciprocal	42	52	1.53
Acnida tamariscina×*Amaranthus retroflexus*	547	0	
Reciprocal	339	374	2.00
A. tamariscina×*A. hybridus* Angol race	1070	0	
Reciprocal	1536	2007	11.72
A. tamariscina×*A. hybridus* Line 58	251	0	
Reciprocal	191	173	1.40
A. tamariscina×*A. hybridus* Line 56	4	0	
Reciprocal	4	3	1.12
A. tamariscina×*A. caudatus* Mexican race	728	0	
Reciprocal	517	410	5.26
A. tamariscina×*A. caudatus* Manchurian race	805	0	
Reciprocal	315	429	6.20
A. tamariscina×*A. Powellii*	17	0	
Reciprocal	—	—	

* Hybrids not studied; but readily obtainable.

results when *A. hybridus* is crossed to *A. spinosus*. These hybrids also have normal flowers but the staminate and pistillate flowers bear no precise positional arrangement with reference to each other. This condition is apparently related to certain factors present in the *A. hybridus* genom which interact only with certain factors in the genom of *A. spinosus* since F_1 hybrids between *caudatus*, *retroflexus*, and *hybridus* show no peculiarities.

Intergeneric crosses of dioecious species of Acnida and monoecious "first type" species of Amaranthus

The results obtained from reciprocal crosses between three different dioecious species and several monoecious first type species are summarized in table 4. All 29 of these intergeneric crosses are consistent in showing that dioeciousness is epistatic over monoeciousness. Fourteen of these crosses were made using the dioecious species as the female parent and these crosses give all female hybrids. The reciprocal crosses, where the monoecious species is the female parent, give ratios of approximately 1 ♀ : 1 ♂. These results are in agreement with CORRENS' (1907) work on Bryonia. His interpretation that the male is the heterogametic sex is likewise true for the three species of Acnida.

All crosses which involve the dioecious species *Acnida cuspidata* and *Acnida tuberculata* give progenies consisting exclusively of normal female hybrids and the reciprocal crosses give 1:1 sex ratios well within the expected deviations. This is not true of certain crosses with *Acnida tamariscina*. The summarized ratios, from certain of those crosses which segregate for males and females, exhibit deviations from a 1:1 ratio much greater than would be expected by chance. Many of the hybrids do not produce flowers and thus there is omitted from table 4 a considerable number of plants whose sex could not be determined. An analysis of individual crosses with consideration given to the non-flowering plants is necessary for an understanding of this problem.

Neuter plants. Plants which do not bear flowers will be designated in this paper as neuter plants. They are indistinguishable from normals in so far as the vegetative parts of a plant are concerned. Neuter plants develop an inflorescence devoid of the actual flowers. The bracts which subtend normal flowers are present but not even minute rudiments are externally visible to indicate that the flower primordium began development. Presumably because the flowers are not present to make nutritional demands on the plant, the individual axes within each flower cluster continue to grow rapidly and a large, crowded, bract-filled inflorescence results. The only fundamental difference is the absence of flowers, as the growth pattern is unchanged.

Neuter plants occur only in the hybrids arising from crosses of the dioecious species *Acnida tamariscina* to any of the monoecious first type species. It must be emphasized that the monoecious species which are used in these crosses are normal in appearance and had been inbred one to three generations. Likewise, *Acnida tamariscina* progeny were grown in considerable numbers without finding any sexual abnormalities and all crosses were made on plants which were inbred one or two generations. It was thought (MURRAY 1938) that the neuter plants were due to an environmental effect on an unstable hybrid complement. An analysis of a larger number of

crosses now shows that the type of segregation depends on the particular Acnida male or female plant used in the cross. Individual plants of either sex give hybrid progenies with no neuter plants, fifty percent neuter plants, or all neuter plants (table 5).

TABLE 5

Types of segregation for neuter plants in reciprocal crosses of Acnida tamariscina to the monoecious "first type" species.

Acnida tamariscina	NUMBER OF PLANTS IDENTIFIED	F_1 PROGENY			D/PE 1:1	χ^2 1:1:2
		♀♀	♂♂	NEUTER		
As female parent:						
Free from neuter	10	1923	0	6*		
Heterozygous for neuter	12	1255	0	1230	.74	
Homozygous for neuter	4	4*	0	292		
As male parent:						
Free from neuter	2	406	510	0	5.09	
Heterozygous for neuter	9	1316	1499	2035	5.09	138.9
Homozygous for neuter	1	1*	0	462		

* Presumably off-pollinations or mechanical mixture of seeds.

The original race of *A. tamariscina* obtained from nature must have been heterozygous for a single dominant factor which induces neuterness when outcrossed to any of the monoecious species. Differences, even in chromosome number, between the monoecious species have no effect on the segregation of neuter in the resulting hybrids. Proof of this comes from the fact that at least three monoecious species with differences in chromosome numbers have been crossed to the same Acnida male or female plant in each of the six classes of segregations found in table 5.

There is a considerable deficiency in the number of neuter progeny in the cross of monoecious by heterozygous male, as one should expect a ratio of 1♀:1♂:2 neuter assuming that the factor is independent of sex. This deficiency is probably not to be ascribed to a lower viability of the neuter plants since in the reciprocal cross the neuter progeny appear with the expected frequency. In these same crosses the frequency of the males is out of proportion to that of the females, although not more so than in the cross where neuters are not present. The neuter-inducing factor inhibits the development of flowers of both sexes although not always in equal proportions. These discrepancies are even greater when one considers the results from any one male plant. The variability encountered includes such extreme ratios as 62♀♀:331♂♂:310 neuter and 424♀♀:307♂♂:548 neuter. A ratio of 271♀♀:278♂♂:408 neuter is much more nearly that expected if there is no linkage with sex. Pollen grains carrying the neuter

factor appear not to function as frequently as those carrying the recessive allele. No satisfactory explanation of these deviations is possible at the present time.

If a female plant of *Acnida tamariscina* known to be free of the neuter factor is crossed to an *A. tamariscina* male known to be heterozygous for the factor, the F_1 male plants segregate for bad pollen. Twelve plants had good pollen and eleven had about 50 percent pollen abortion. Likewise, if a female plant known to be homozygous for the neuter factor is crossed to a male plant known to be heterozygous, the F_1 males segregate. Twenty-seven F_1 plants had good pollen and 12 plants had about 50 percent pollen abortion. These records indicate that the neuter-inducing factor when heterozygous is associated with 50 percent pollen abortion and probably with 50 percent ovule sterility. A cytological study has not yet been made to ascertain whether a chromosomal change is actually present.

Flower color. The species *Acnida tamariscina* exhibits variations in plant color and an attempt was made to study these in the hope of obtaining a sex-linked character. Crosses of entirely green-stemmed plants to brilliant red-stemmed ones gave apparently heterozygous intermediate red-stemmed plants. When one of these heterozygous females is crossed to a monoecious species, linkage with the neuter-inducing factor is found. The results are given in tabular form below:

Red ♀	Green ♀	Red neuter	Green neuter
1	322	344	4
182	5	4	153

Since these are essentially backcross progenies, the two linkage phases may be added together in the calculation of the percent of recombination (1.4 percent).

The results of a second generation analysis of plant color in the species *Acnida tamariscina* are difficult to interpret. They suggest that the interaction of two autosomal genes are responsible for the type of plant color. It has been shown that one of these genes is closely linked to the neuter-inducing factor and that the latter is probably related to a chromosomal change. While an unknown translocation or inversion by itself ought not to disturb the ratios, partial linkage with a lethal would. This seems to be true. The neuter factor may be considered an autosomal factor since the closely linked color factor does not show sex linkage.

Neuter hybrids occasionally have small chimeras in the inflorescence and these areas bear either normal staminate or pistillate flowers. In those families where the neuter hybrids are all modified females these chimeras have pistillate flowers. In other families, where the neuter hybrids may be either modified males or females, some of the plants have pistillate

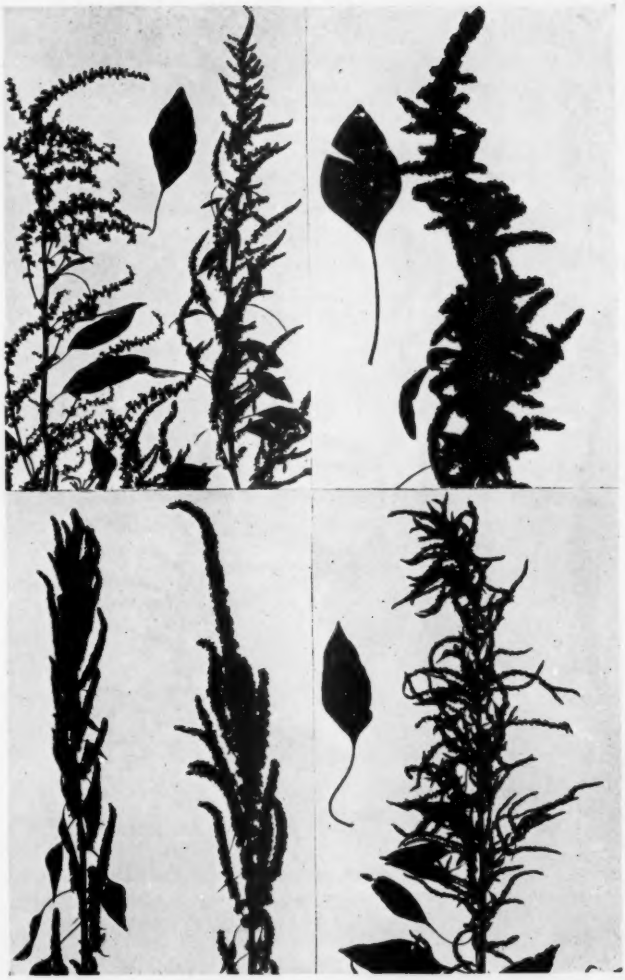

FIGURE 2. a (upper left).—Normal *Acnida tamariscina* (dioecious). Male at left, female at right. b (upper right).—*Amaranthus hybridus* Angol race, a monoecious first type species. c (lower left).—F_1 female (left) and male (right) hybrids resulting from the cross of *Amaranthus hybridus* Angol race by *Acnida tamariscina* male. d (lower right).—F_1 neuter hybrid resulting from the same cross.

FIGURE 3. a (upper left).— F_1 neuter hybrid resulting from the cross of *Amaranthus hybridus* Angol race by *Acnida tamariscina* male. Arrow points to chimera of normal male flowers. b (upper right).—Viable F_1 male hybrid resulting from cross of *Acnida tamariscina* by *Amaranthus spinosus*. Picture includes only one branch of a plant five feet tall. c (center).—Barely viable F_1 male hybrid resulting from the same cross. Note the size of this complete plant ($1\frac{1}{4}$ inches tall) of approximately the same age as the plant figured in b. d (lower left).—*Amaranthus spinosus*, a monoecious second type species. Arrow indicates division line between female (below) and male flower clusters (above). Note lighter V-shaped area on leaf due to presence of a dominant gene. e (lower right).—F_1 monoecious hybrid resulting from cross of *Amaranthus hybridus* Angol race by *Amaranthus spinosus*. No definite positional arrangement of the staminate and pistillate flowers.

chimeras, others staminate ones. The sex of the F_1 hybrids is quite stable in that staminate and pistillate flowers are rarely found on the same plant. The only observed exceptions were a few neuter plants which had both staminate and pistillate chimeras. Figure 3a illustrates a striking example of a large chimera where normal male development occurs on one side of a neuter plant. A cytological study of the male flowers of this chimera showed that development was halted before division I. It was therefore impossible to learn whether one or more chromosomes had been lost somatically.

One family not included in table 4 or 5 was a cross of *Acnida tamariscina* by *Amaranthus hybridus* Angol race. This cross gave 3 female to 11 monoecious plants. The flower arrangement of the monoecious hybrids was identical to that of the monoecious parent and somatic chromosome counts on one monoecious hybrid did not reveal any deviation from the expected number of 32 chromosomes. Apparently the particular Acnida female used in the cross was heterozygous for a mutation affecting sex, but unfortunately no other crosses were made on the same plant and this type of cross has not reappeared. Since part of these plants were grown in the greenhouse and part in the experimental plot, it is unlikely that this change was due solely to environmental causes. All the hybrid plants were pollinated but produced no fertile seed.

BRESSMAN (1934) has noted a high percentage of non-flowering (neuter) hop plants in certain cultures. Whether there is any real similarity between the neuter plants found in Acnida and hops cannot be determined from the published facts.

Intergeneric crosses of dioecious species of Acnida and monoecious "second type" species of Amaranthus

Amaranthus spinosus does not hybridize as readily with the dioecious species as do the monoecious first type species. The first progenies of these hybrids consisted entirely of small plants which usually died in the seedling stage. The fourth cross in table 6 is an example. Twenty-two plants out of 350 survived until they flowered and these grew very slowly for five months and never attained a height of more than two inches. This non-viability is associated with certain crosses and not with others. The first three families (table 6) indicate that progenies may be obtained consisting of all non-viable seedlings, 50 percent non-viable seedlings, or all viable seedlings. The dominant factor responsible for non-viability in the hybrids is probably carried in the dioecious species, *Acnida tamariscina*, since the *Amaranthus spinosus* plants came from a line inbred for two generations. In fact, this non-viability factor may be identical with the factor which induces the development of neuter hybrids in crosses with the monoecious first type species.

Disregarding non-viable progeny, males predominate in the cross dioecious by monoecious where all females would be expected if the monoecious parent had been one of the first type species. In addition to the males a limited number of monoecious plants are produced. In the reciprocal cross, males also predominate. Apparently male-determining factors present in the monoecious genom are epistatic over female-determining factors present in the dioecious genom.

TABLE 6

F_1 hybridization results of intergeneric crosses of dioecious Acnida species and Amaranthus spinosus, the monoecious "second type" species.

CROSS	♀♀	♂♂	MONOECIOUS	REMARKS
Acnida tamariscina×*Amaranthus spinosus*	0	347	3	No loss of seedlings
Acnida tamariscina×*Amaranthus spinosus*	6	207	41	50% loss of seedlings
Acnida tamariscina×*Amaranthus spinosus*	0	3	0	±100% loss of seedlings
Amaranthus spinosus×*Acnida tamariscina*	1	17	4	All are type usually dying as seedlings
Amaranthus spinosus×*Acnida tamariscina*	0	186	4	No loss
Amaranthus spinosus×*Acnida cuspidata*	0	3	3	No loss
Amaranthus spinosus×*Acnida tuberculata*	0	52	7	No loss

It is difficult to know how much significance should be attached to the occurrence of the monoecious plants enumerated in table 6. Pistillate flowers are developed sporadically in the lowermost axils of these plants and vary in number from a few flowers to a maximum not exceeding 5 percent of the total. While a hybrid with 10,000 staminate flowers and only 25 pistillate flowers may be called monoecious, it is not strictly comparable to its monoecious parent which has an equal number of staminate and pistillate flowers. A study of phenotypic expression gives one the impression that factors which localize the development of the staminate flowers at the ends of the inflorescence branches in *A. spinosus* are lax in their effects in the hybrids and allow the production of staminate flowers throughout the plant with the possible exception of the lowermost axils which ordinarily do not produce flowers. Stem cuttings taken from the base of a hybrid plant, sometimes at least, produce an abundance of female flowers. The few female plants are most difficult to explain on a genotypic basis. Furthermore, the three monoecious hybrids found in the cross of *Amaranthus spinosus* by *Acnida cuspidata* approach the normal monoecious parent much more closely in phenotypic expression. One of these plants was a tetraploid.

Backcross of F_1 hybrids obtained from "♂" to both parental types

The primary aim of this work was to find a hybrid combination of stable dioecious and monoecious species which would be fertile enough to enable

one to study backcross progenies. The dioecious species of Acnida have a haploid chromosome number of 16, the monoecious species of Amaranthus 16 or 17. This indicates that *Amaranthus hybridus* and *caudatus* are more closely related to Acnida than are *retroflexus* or *spinosus*. Yet all cross readily with Acnida to give F_1 hybrids. All F_1 hybrid combinations exhibit about 99 percent pollen abortion and the ovule sterility must be equally as great, although this is more difficult to estimate. A cytological examination of the microsporocytes of one intergeneric hybrid showed that chromosome distribution in the first division is very unequal and is even more unequal in the second division. This fact by itself could account for the high sterility. It is interesting that fertility in the hybrids is restored concurrently with the doubling of the chromosome complement. This indicates that failure to synapse properly is probably the major cause of this sterility.

TABLE 7

Backcross of F_1 hybrid females (derived from intergeneric crosses) to parental types.

CROSS	♀♀	♂♂	NEUTER	MONOECIOUS		D/PE
				NORMAL	SPORADIC	1:1
Plot E and F. Backcross to dioecious parent						
F_1 (*Acnida tamariscina*×*Amaranthus hybridus* Angol race)×*A. tamariscina*	488	520	15	0	6	1.50
F_1 (*Acnida tamariscina*×*Amaranthus retroflexus*)×*A. tamariscina*	37	52	15	0	0	2.52
Plot A. Backcross to monoecious parent						
F_1 (*Amaranthus hybridus* Angol race×*Acnida tamariscina*)×*A. hybridus* Angol race	255	14	3	7	9	
F_1 (*Acnida tamariscina*×*Amaranthus hybridus* Angol race)×*A. hybridus* Angol race	1558	328	114	42	40	
Control. Normal *Acnida tamariscina*×*A. hybridus* Angol race	125	5	14	0	0	
Plot C. Backcross to monoecious parent						
F_1 (*Acnida tamariscina*×*Amaranthus retroflexus*)×*A. retroflexus*	54	0	0	6	0	
F_1 (*Amaranthus retroflexus*×*Acnida tamariscina*)×*A. retroflexus*	263	0	1	3	1	
F_1 (*Amaranthus retroflexus*×*Acnida cuspidata*)×*A. retroflexus*	6	0	0	0	0	

Use of the pollen from the male hybrids is not feasible, because the flowers usually do not open sufficiently to protrude the anthers, and the anthers usually fail to dehisce. As preliminary backcrossing attempts using the female hybrids yielded so few seeds per pollination, the use of isolation plots was necessary. Ten to 25 female hybrids were planted in each plot

together with the pollen producer which was either the dioecious or the monoecious parent. As noted previously, new flowers are continually produced in unpollinated inflorescences or where the seed set is poor. Consequently, a large hybrid plant at the end of the season may have had as many as a hundred thousand flowers and so would yield some seed despite the high sterility. Results from these backcross progenies are summarized in table 7.

Backcrosses to the dioecious parent (plots E and F) in the two cases studied give ratios of 1 ♀ : 1 ♂ with no more than the usual deviation. This result was expected because these are backcrosses to the epistatic sex condition. The neuter plants are due to the dominant factor carried in the dioecious parent, *Acnida tamariscina*. No significance can be attached to the proportion of neuter hybrids since several male plants were used as pollen producers. Part of these may have been free from this factor, part heterozygous for it, and part homozygous for it.

The results from backcrossing the F_1 female hybrids to the monoecious parent are shown in table 7, plot C. The results are essentially the same regardless of which way the F_1 cross was made, but the high proportion of females and low proportion of monoecious plants was not anticipated. If the segregation of chromosomes in the hybrid was completely random, gametes containing all possible combinations of the two chromosome complements should be secured. While many of these might be lethal, one should expect at least three kinds of functional gametes; namely, those having an entire genom originally obtained from the dioecious parent, those having an entire genom originally obtained from the monoecious parent, and those having both genoms. The first type of hybrid gamete after fertilization by a monoecious gamete should reconstitute the F_1 hybrid female, the second should reconstitute the monoecious parent and the third should produce a triploid plant with two monoecious genoms and one dioecious female genom. One can be reasonably certain that the third type of gamete is functional, otherwise doubling the chromosome complement of these hybrids ought not to restore fertility. The second type of gamete must be formed very infrequently because the monoecious plants, which occur, are sterile and phenotypically unlike the parental monoecious species. One cannot be sure about the first type of gamete since the phenotypic appearance of the resulting F_1 hybrids might be indistinguishable from the triploids. These second generation plants are remarkably uniform in all characters and while similar in appearance to the F_1 hybrids, their pubescence, leaf shape and habit approach more closely the characters of the normal monoecious parent, *Amaranthus retroflexus*. Somatic chromosome counts were made for 16 plants selected at random from the cross F_1 (*Acnida tamariscina* × *Amaranthus retroflexus*) × *Amaranthus retroflexus*.

All plants had the triploid number of 50 chromosomes which would be attained by the combination of one dioecious Acnida genom of 16 chromosomes with two monoecious Amaranthus genoms of 17 chromosomes each. Likewise, nine plants from the cross F_1 (*Acnida tamariscina* × *Amaranthus hybridus*) × *Acnida tamariscina* have the expected triploid number of chromosomes, $(16+16+16)=48$. This is proof that the F_1 hybrids produce only one principal type of viable gamete and that this gamete contains both genoms.

Progenies derived from plot A crosses differ from plot C crosses in having a considerable number of male and neuter plants. There must have been some dioecious pollen present in this plot since the control had five males. Chromosome counts of several females selected at random show that these plants have the expected triploid number of 48 chromosomes obtained from backcrossing to the monoecious parent. However, one neuter and one male definitely had 48 chromosomes which could be attained from backcrossing to the dioecious parent. Thus, the male and neuter plants found in these families probably should be disregarded. An equal number of the females should have been due to pollination with dioecious pollen. The corrected figures for plot A are 241 ♀ ♀ : 16 monoecious or a ratio of 15:1 and 1230 ♀ ♀ : 82 monoecious or a ratio of 15:1. In these two crosses the F_1 hybrid female plants came from reciprocal crosses. Plot C has 323 ♀ ♀ : 10 monoecious or a ratio of 31:1.

It is difficult to evaluate the significance of these ratios and the presence of the monoecious plants. They have a near triploid constitution but this may not be a balanced complement. In so far as the vegetative parts of the plant are concerned, monoecious plants are indistinguishable from triploid females. The production of stamens is limited to the first flower of each flower cluster exactly as in the monoecious first type species. Ovary development may or may not be inhibited in these staminate flowers and this varies on the same plant. Some plants have all the first flowers of each flower cluster staminate or perfect (called normal monoecious); others have only a few of the first flowers staminate or perfect (called sporadic monoecious). With so much variability of expression, this enumeration may have little significance.

Backcross of F_1 colchicine-induced tetraploid hybrids obtained from "3" to both parental types

A 0.25 percent aqueous solution of colchicine applied to the growing points induced tetraploid chimeras on several female hybrids and these areas with restored fertility were backcrossed to the parental species. The results are summarized in table 8.

TABLE 8

Backcross of F_1 hybrid colchicine-induced tetraploid females (derived from intergeneric crosses) to parental types.

CROSS	♀♀	♂♂	D./P.E. 1:1	D./P.E. 1:2
Backcross to dioecious parent:				
F_1 (*Acnida tamariscina*×*Amaranthus caudatus* Mexican race)×*A. tamariscina*	265	311	2.8	
F_1 (*Acnida tamariscina*×*Amaranthus hybridus* Angol race)×*A. tamariscina*	26	28	0.4	
F_1 (*Amaranthus hybridus* Angol race×*Acnida tamariscina*)×*A. tamariscina*	50	49	0.3	
F_1 (*Acnida tamariscina*×*Amaranthus caudatus* Manchurian race ×*A. tamariscina*	40	86	6.0	0.6
F_1 (*Amaranthus caudatus* Manchurian race×*Acnida tamariscina*)×*A. tamariscina*	108	114	0.6	
Backcross to monoecious parent:				
F_1 (*Acnida tamariscina*×*Amaranthus caudatus* Mexican race)×*A. caudatus* Mexican race	238	0		
F_1 (*Acnida tamariscina*×*Amaranthus caudatus* Manchurian race)×*A. caudatus* Manchurian race	69	0		
F_1 (*Amaranthus caudatus* Manchurian race×*Acnida tamariscina*)×*A. caudatus* Manchurian race	6	0		

Backcrosses to the dioecious parent gave ratios of 1 ♀ : 1 ♂ and these plants have the expected triploid number of 48 chromosomes. Progenies derived from backcrosses to the monoecious parent consist exclusively of female plants and nineteen plants selected at random have the expected triploid number of 48 chromosomes. These backcross progenies are similar to the untreated backcrosses except that monoecious plants are not found.

DISCUSSION

It has been shown that a cross of an Acnida female of a dioecious species to a monoecious "first type" species gives only females and that the reciprocal cross gives an equality of males and females. Since the male is the heterogametic sex, the sex mechanism of these three dioecious species is, therefore, of the XY type. These crosses also give a direct comparison of the female genom X+15A to the male genom of Y+15A as the monoecious genom is identical in both cases. If the assumption is made that the autosomes exhibit random distribution in the formation of gametes and are either homozygous for all sex factors or lack of such factors, one may conclude that the sex chromosome pair (XY) contains the differential genic mechanism which initiates the development of maleness or femaleness in an individual sporophyte under normal conditions. If the auto-

somes contain sex factors and are not homozygous for them, segregation should occur in the inbred lines of the dioecious species as well as in the crosses to the related dioecious and monoecious species. However, there is no evidence of segregation either within the species or in the interspecific hybrids. If random distribution of the autosomes does not occur, abnormal ratios for two autosomal genes should be secured, but are not. This interpretation cannot be considered absolute proof that the Y chromosome carries the male-determining factors. However, if the Drosophila type of sex inheritance were true of Acnida, one should expect the XXXY ($_3X+_4A$) tetraploid Acnida males to be intersexes.

Since intersexes, hermaphroditic plants or sexual abnormalities are not found in the second generation progeny of an interspecific cross of two different dioecious species, the genic sex mechanism is apparently identical in both species. The interpretation is made that such exact similarity would be unlikely if sex were due to the interaction of a large number of genes.

TABLE 9

Genotypic balance in relation to sex.

DIPLOID	TRIPLOID	TETRAPLOID
Pure Species:		
d d = ♀		*d d d d* = ♀
D d = ♂		*D d d d* = ♂
		D D d d = ♂
m 1 *m* 1 = mon.$_1$		*m* 1 *m* 1 *m* 1 *m* 1 = mon.$_1$
m 2 *m* 2 = mon.$_2$		
Hybrids:		
d *m* 1 = ♀	*d m* 1 *m* 1 = ♀	*d d m* 1 *m* 1 = ♀
D m 1 = ♂	*d d m* 1 = ♀	*D D m* 1 *m* 1 = ♂
d m 2 = ♂	*D d m* 1 = ♂	
D m 2 = ♂	*d m* 1 *m* 2 = ♂ or mon.*	*d d m* 2 *m* 2 = mon.$_2$**
m 1 *m* 2 = mon.$_1$*		

Where *d* = ♀ genom of dioecious species; *D* = ♂ genom of dioecious species; *m* 1 = genom of monoecious species first type; *m* 2 = genom of monoecious species second type

* Sometimes intermediate.

** Only one plant observed.

The dioecious and monoecious "first type" species used in this work do not enable one to study directly the genetic basis of the evolution of sex. However, it is possible to compare several combinations of genoms and their relation to sex (table 9).

It is shown, in the present work, that males are formed if one male genom of Acnida is present with one monoecious first type genom of Amaranthus, and that females are formed when one female genom of Acnida is present with one or two monoecious genoms of Amaranthus and only in the absence of a male genom. MURRAY (unpublished) has shown, from a study of tetraploids of *Acnida tamariscina*, that malesness is induced when one male genom is present with one or even three female genoms.

The combination of a single female genom of Acnida with a genom of the monoecious second type species of Amaranthus usually results in maleness although occasionally the males produce a few pistillate flowers near the base of the plant. In addition a few females have been observed. It has been assumed that factors inducing maleness in the second type monoecious plant are generally epistatic to the factors inducing femaleness. When a female genom, a monoecious first type genom, and a monoecious second type genom are combined in a triploid, the result is either a male plant or a monoecious one without any precise positional arrangement of the pistillate and staminate flowers. This peculiar type of monoecism has been observed in these triploid progeny only when the monoecious first type genom is that of *Amaranthus hybridus* Angol race. The same result is obtained in a diploid hybrid when a genom of the Angol race is combined with one of the second type of monoecism. Other species of the first type of monoecious arrangement are completely epistatic over the second type; and the addition of a single female genom of *Acnida tamariscina* to these diploids results in the formation of triploid males. Whether these phenotypic effects are due to many or only a few genes cannot be stated.

The intergeneric F_1 hybrids between dioecious and monoecious first type species produce only one type of functional gamete and this contains the diploid number of chromosomes including both parental genoms. These gametes probably arise from the reconstitution of the chromosomes into one nucleus after an abortive first division. MANGELSDORF and REEVES (1939) have observed the same phenomenon in the intergeneric hybrids of Zea×Tripsacum where the only functional gametes are those possessing both genoms.

It has been shown that the presence of barely viable hybrids in the crosses of *Acnida tamariscina* to *Amaranthus spinosus* are found only in certain crosses and not in others and that their presence is probably conditioned by a single factor carried in the dioecious species. This phenomenon may be of general occurrence in wide crosses. MANGELSDORF and REEVES (1939, pp. 89, 103) have reported vigorous and dwarf F_1 hybrids in the intergeneric crosses of Zea×Tripsacum. They also cite examples in Fragaria hybrids and in sorghum-sugarcane hybrids. Since these species

are probably highly heterozygous, the same type of explanation may very well apply.

The presence of neuter hybrids in certain crosses between *Acnida tamariscina* and all monoecious first type Amaranthus species constitutes a new kind of isolation mechanism. These species will hybridize readily if growing in the same area, but hybridization could not be carried beyond the first generation since in certain lines all the F_1 hybrids are neuter plants. This mechanism is supplemented by the low degree of fertility of the hybrids which flower. It has been shown that either *Acnida tamariscina* males or females may carry the dominant neuter-inducing factor in either a homozygous or heterozygous condition, and appear phenotypically normal. Neuter hybrids are obtained only on outcrossing to one of the monoecious first type species. The factor is shown to be linked to a color gene with 1.4 percent crossing over, and when heterozygous induces 50 percent pollen abortion and presumably 50 percent ovule sterility. This suggests that the factor is probably related to, or due to, a translocation or an inversion rather than a point mutation. Phenotypically neuterness results in the suppression of flowers of either sex, although not always in the same proportion. Any direct relation to the mechanism of sex determination or evolution of sex is not apparent.

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SUMMARY

In this study of the genetics of sex determination, three dioecious species of the genus Acnida and five monoecious species (eight races) of the genus Amaranthus, family Amaranthaceae, have been used. The monoecious species exhibit two types of distribution of the male and female flowers, although the growth pattern of the inflorescence of all species, regardless of sex, is identical.

Included in this paper are the results of an investigation of 29 interspecific and 33 intergeneric F_1 hybrids; the production of tetraploid races in one dioecious and two monoecious species; the creation of five amphidiploid races; and an analysis of about 6000 second generation progeny obtained from backcrossing F_1 intergeneric hybrids to the parental species. Altogether, over 50,000 plants were used.

Genetical data indicate that the male Acnida plant is heterogametic. The hypothesis is advanced that the XY chromosome pair in the dioecious

species carries the differential sex factors and that the autosomes, if they carry sex factors, are homozygous for them not only within the species, but also in different species.

Several diploid, triploid, and tetraploid combinations of dioecious and monoecious genoms have been studied. These data show that the complex of sex factors in the Y chromosome are epistatic to those in the X chromosome and to those in a monoecious genom. Likewise, the factor, or factors, in the X chromosome are epistatic to those in the monoecious "first type" genom but are hypostatic to those in the monoecious "second type" genom.

Neuter plants, wherein the flower primordia fail to develop, occur among certain F_1 intergeneric hybrids. This condition is shown to be due to a single dominant autosomal factor present in the species *Acnida tamariscina* and *only expressed* when outcrossed to any of the monoecious "first type" species. The neuter factor is linked to a color factor (1.4 percent C.O.) and when heterozygous is associated with 50 percent pollen abortion. This suggests that the factor may be carried on a translocated or inverted region. The development of flowers of both sexes is inhibited by the neuter phenotype, so that there is probably no direct relationship to the mechanism of sex determination.

The hybridization of certain *Acnida tamariscina* plants with *Amaranthus spinosus* results in the production of barely viable hybrids. Progenies may contain all non-viable, 50 percent non-viable, or all viable hybrids. A single dominant factor, perhaps identical to the neuter-inducing factor, apparently conditions the production of barely viable hybrids. The neuter-inducing factor and also the non-viability factor (if different) are tentatively cited as new physiological types of isolation mechanisms.

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